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## PROCEEDINGS OF SYMPOSIUM ON TUBERCULOSIS IN INFANCY AND CHILDHOOD

*National Jewish Hospital at Denver*

November 9-12, 1955

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PROCEEDINGS

OF

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*at Denver*

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## PREFACE

In the summer of 1954, several of our physicians suggested that National Jewish Hospital at Denver sponsor a symposium on tuberculosis in infancy and childhood. They proposed that some of the world's eminent authorities on this subject be invited to our institution for this purpose.

Their plan fitted in perfectly with the Hospital's fourfold program of treatment, research, education, and rehabilitation. The Symposium would give our staff the benefit of the latest thinking on the management of pediatric tuberculosis and on research in this area. By publishing the full record of the papers and the discussion by the distinguished participants, the educational function of our Hospital would be well served.

With the holding of this Symposium, our institution had come a long way. It opened in 1899 with one building, one doctor, and one nurse. The first patient was a Catholic girl from Chicago. The Hospital's objective was to create a first-class institution for the treatment of victims of tuberculosis who were unable to meet the enormous cost of long-term private care, without respect to race, creed, or national origin.

Then, as now, the Hospital's motto was "None may enter who can pay; none can pay who enter." During the past fifty-seven years, our patients have received more than 4,000,000 days of care, completely without charge. We like to think we give care at least equal to that which the patient who is able to pay receives.

From its humble beginnings, National Jewish Hospital at Denver has grown steadily. In 1919 a modest research program was initiated. It has since grown to significant proportions. In 1938 a professional social service program was undertaken.

Today the institution occupies fourteen buildings, has a full-time staff of about 400, and numbers 150 physicians on its resident, consulting, visiting, and research staff.

When it was suggested that our institution play host to the Symposium, our board of trustees enthusiastically agreed. Recognizing that the function, the responsibility, of the Hospital is something more than the treatment of individual patients, the board appropriated a substantial sum to meet the entire cost of the Symposium. Thus, it was possible to have the valued contributions of the eminent foreign participants, many of whom might otherwise have been unable to come to Denver.

In addition to the 25 scientists who made formal presentations, approximately 125 specialists attended the Symposium by invitation. Unfortunately, but obviously, it was impossible to invite all of the leading authorities in the field.

For those who helped in one way or another to make the Symposium a reality this was a most exciting experience.

Now these proceedings—the fruit of more than two years of hard work—are in your hands. May you find them a useful contribution to the literature.

June 12, 1956

PHILIP HOUTZ
Executive Director
National Jewish Hospital at Denver

## FOREWORD

The medical science motto of the National Jewish Hospital at Denver might well be, "From the Intimate Metabolism of the Parasite to the Psyche of the Host," because all of the disciplines of contemporary medical science are drawn upon in the field of tuberculosis. It is our conviction that this disease, in its many aspects, provides a broad basis for teaching the fundamentals of medicine and many of its ancillary disciplines.

It is clear why this view is not widely held. The traditional centers of care for the tuberculous, unlike those which treat other important plagues of mankind, have been geographically remote from the great teaching centers of medicine, isolated, as it were, in the sanitary air of the countryside. This tradition, which fostered a resigned serenity inimical to intensive intellectual activity, has been partially broken during the past two decades, first, by the increasingly important role played by excisional thoracic surgery and, lately, by the spectacular developments in antimicrobial chemotherapy.

The inevitable result of more specific, more successful therapy has been a reawakening of general academic interest in the mechanisms of tuberculous infection and disease and their control. Even more striking are the growing numbers of points of controversy concerning methods of prevention and treatment of tuberculosis.

This first international *Symposium on Tuberculosis in Infancy and Childhood* was a natural consequence of the present unsettled climate of opinion in the field of pediatric tuberculosis, an important responsibility of our Hospital. It seemed appropriate that such a symposium should be held in Denver. This is one of the outstanding chest disease teaching centers of the world, where tuberculosis, because it so often manifests itself as disease in the chest, has long been a special subject of research and treatment.

James B. Conant has suggested that science consists of any activity which "reduces the degree of empiricism" in any field of human endeavor. The several distinguished investigators who presented papers at this conference were invited because they had revealed an uncommon skill in analyzing pathogenetic processes and the dynamics of therapy. Their contributions were aimed at decreasing the degree of empiricism in efforts to prevent and to treat tuberculosis. For this very reason theoretical considerations were given priority over what is often referred to as *clinical experience* or statistical analyses of *practical results*.

Many subscribe to the view that the productive scientist must have convictions, even prejudices, to formulate hypotheses on which to base his objective inquiries. Accordingly, every effort was made to foster opportunities for frank unfettered expressions of opinion and unproved theory in planning and conducting this Symposium. And the editors have sought to preserve this freedom in this complete publication of its proceedings.

Under these circumstances it would be wrong to pretend that the idea of holding this conference was not based upon some convictions and preconceptions of its planners:

First, recent advances in effectiveness of treatment with antimicrobial agents—isoniazid, in particular—have indicated that wider clinical application of diagnostic tuberculin testing and chemotherapy of the infection in specific age groups (infancy and puberty) should decrease dramatically the incidence of the serious manifestations of tuberculosis in infancy and perhaps even in subsequent adulthood.

Second, the effectiveness of antimicrobial therapy in host-parasite relationships, and particularly in tuberculosis, depends upon several considerations which, in our opinion, have not received wide enough recognition among pediatricians or tuberculosis specialists. For example, there appears to be a "physiologic," as distinguished from a "genetic," susceptibility of parasites to the bactericidal activities of the available drugs, particularly in relation to bacterial multiplication or dormancy in different lesions, even in the same host. Thus, every effort should be made toward an integration of immunologic phenomena, in the broadest sense, both with the mechanisms and with the limitations of chemotherapy.

Third, we believe that long-term prevention and treatment of disease in youngsters requires a preoccupation with emotional development which is perhaps beyond the classical confines but not beyond the contemporary responsibilities of medical science.

It was not possible, of course, to cover all aspects of these problems, but we hope that the reader of these proceedings will gain some insight into their practical applications by studying what we were privileged to learn from our eminent guests.

June 9, 1956

Gardner Middlebrook,
Director of Research and Laboratories
Sidney H. Dressler,
Medical Director
National Jewish Hospital at Denver

## CONTENTS


PREFACE ..... i

FOREWORD ..... iii

SESSION 1

Childhood Tuberculosis. The Problem. RENÉ J. DUBOS ..... 1
Morphologic Analysis of Fatal Tuberculosis in Children. With Some Comments on Endogenous Exacerbation and Tuberculous Meningitis. KORNEL L. TERPLAN ..... 7
Dynamic Aspects of the Pathology and Bacteriology of Tuberculous Lesions. GEORGES CANETTI ..... 13
*General Discussion* ..... 22

SESSION 2

Vaccination Against Tuberculosis. Clinical Aspects. LARS STRÖM ..... 28
Usual and Unusual Reactions to BCG Inoculation in Children. HANS JACOB USTVEDT ..... 32
BCG and Vole Vaccinations. K. NEVILLE IRVINE ..... 43
*General Discussion* ..... 50

SESSION 3

Immunopathology of Tuberculosis. SIDNEY RAFFEL ..... 60
Pathogenicity of Isoniazid-Resistant Tubercle Bacilli and Prophylaxis of Tuberculosis in Children. NOËL RIST ..... 75
*General Discussion* ..... 90
Dynamics of Antituberculous Chemotherapy. WALSH MCDERMOTT, ROBERT M. MCCUNE, JR., AND RALPH TOMPSETT ..... 100
The Bactericidal Activities of Antituberculous Drugs. D. A. MITCHISON and J. B. SELKON ..... 109
*General Discussion* ..... 116
Complications of Skeletal Tuberculosis and Its Treatment. CHARLES H. LACK ..... 124
*General Discussion* ..... 134

SESSION 4

Some Observations on the Utility of Simian Pulmonary Tuberculosis in Defining the Therapeutic Potentialities of Isoniazid. L. H. SCHMIDT ..... 138
*General Discussion* ..... 153
Tuberculous Cutaneous Complexes in Children. R. V. PLATOU AND R. H. LENNOX ..... 160
*General Discussion* ..... 169

Nutrition and Tuberculosis in the Adolescent. Joseph A. Johnston . . 173
*General Discussion* . . . . . 183

Dinner Meeting

Changing Attitudes Toward the Therapy of Tuberculosis. Sidney H. Dressler . . . . . 188

Session 5

Systematic Treatment of Primary Tuberculosis. Robert Debré . . . . 191
Effects of Isoniazid on Allergy. With Some Additional Observations in Children Vaccinated with BCG. C. Choremis, D. Liacacos, I. Allamanis, D. Zoumboulakis, and H. Kourti . . . . . 197
Cortisone and Corticotropin in the Treatment of Tuberculosis in Infancy and Childhood. Cesare Cocchi . . . . . 209
*General Discussion* . . . . . 216
Present Method and Results of Treatment of Tuberculous Meningitis. Robert Debré and H. E. Brissaud . . . . . 221
The Management of Special Problems in the Chemotherapy of Tuberculosis in Infants and Children. José E. Sifontes . . . . . 225
Correlation of *in Vitro* Susceptibility of Tubercle Bacilli in Patients with Miliary and Meningeal Tuberculosis with Their Response to Therapy. Hattie E. Alexander, Douglas S. Damrosch, and Catherine Ellis . . . . . 232
*General Discussion* . . . . . 240

Session 6

The Course and Prognosis of Endobronchial Tuberculosis in Children. Edith M. Lincoln, Leonard C. Harris, Somchai Bovornkitti, and Rosario Carretero . . . . . 246
Experiences with Intra- and Extraluminal Bronchial Tuberculous Lesions. Robert H. High and Waldo E. Nelson . . . . . 256
Bronchoscopy in Primary Tuberculosis of Childhood. Herman I. Laff, Mauricio Goldberg, and William F. Russell, Jr. . . . . 267
*General Discussion* . . . . . 278
Views in Perspective. Bela Schick . . . . . 290

Summary Session . . . . . 297

## Session 1

Wednesday morning, November 9, 1955

EMANUEL FRIEDMAN, *Honorary Chairman*

RENÉ J. DUBOS, *Moderator*

# CHILDHOOD TUBERCULOSIS

### The Problem

RENÉ J. DUBOS[1]

When I first received the tentative program of our symposium with my name linked to a paper entitled "The Problem," a story told of Gertrude Stein immediately came to mind. According to the story, Gertrude Stein on her sick bed was heard crying out repeatedly in an anguished voice, "What is the answer? What is the answer?" "Do not torment yourself," responded her companion, Alice B. Toklas, "there is no answer." To which Gertrude Stein retorted, "But if there is no answer, what, then, is the question?"

Like Gertrude Stein, I am unclear as to the nature of the question that we have been called here to answer. But one aspect of the problem, at least, needs no explanation, namely, the continuing importance of tuberculosis in our society. Only two figures need be quoted to enlighten those who listen to the irresponsible statements that the disease has been conquered, all but wiped out in the modern world. In the United States alone, 15,000 persons will die of tuberculosis this year, whereas the death rate of poliomyelitis is likely to be around 1,500, a tenfold difference. A similar contrast comes to light when one considers the relative morbidity rates of the two diseases. Twenty thousand persons will be seriously ill with poliomyelitis this year in the United States, and at least 200,000 with tuberculosis. Here again, a tenfold difference. Even when looked for with the most optimistic eyes, the conquest of tuberculosis is not yet in sight.

Although the conveners of the symposium have instructed me to open your deliberations with a statement of "The Problem," they have not listed any paper that would conclude the proceedings by providing "The Answer." This omission was wise, for it is unlikely that any one single formula could be found at the present time to deal with the control of childhood tuberculosis. Differences in the ecological situations, and in the extent of the control that the community can afford to achieve, make of childhood tuberculosis not one problem, but many problems, with as many different solutions.

I realize that our role here is to concern ourselves with the future, and not to dwell in history. But I also believe that, in the words of the Queen in *Alice in Wonderland*, "It is a poor kind of memory that works only backward." Allow

[1] Rockefeller Institute for Medical Research, New York, New York.

me for a minute, therefore, to consider history both backward and forward in an attempt to gain a long-range perspective of our problem.

Consider for example the question so often debated in the past: Is the primary infection that is contracted during childhood a blessing because it endows the infected individual with greater resistance against disease later in life; or is it a tragedy because it constitutes the seed from which clinical tuberculosis may eventually develop? In my opinion, this apparently simple alternative ignores many aspects of the problem, for the answer must be conditioned by epidemiological considerations.

The view that the primary infection of childhood is a useful asset gained ascendancy at a time when heavy exposure to tubercle bacilli was an almost constant feature of everyday life, and when the mode of living rendered a great majority of the people highly susceptible to tuberculosis. There was then a widespread belief in the existence of a "golden age of resistance" to tuberculosis, based on the observation that, on the whole, the disease rarely progressed to a dangerous point in children below the age of puberty. Yet these same children were known to develop some degree of antituberculous immunity as a result of infection contracted during the early adolescent years. We are now inclined to believe that our predecessors overestimated the resistance of the school-age population. Nevertheless, it is probably true that it was of statistical advantage for the dwellers of industrialized cities around 1900 to have acquired some immunity through the primary infection contracted in childhood since they were heavily exposed to infection for the rest of their lives in crowded factories, shops, and tenements.

As we know, the epidemiological situation is very different in much of the Western World today. Exposure to heavy infection is becoming rare, and the standard of living under ordinary circumstances is adequate to secure for most normal persons a fair degree of natural resistance to tuberculosis. Antituberculous immunity, while still useful, is no longer essential for survival in our communities. Furthermore, there is much evidence that the immunity resulting from primary infection in childhood is bought at a great price. The risks that it entails are not small, since tuberculous infections that remain latent during the early years of life may flare up in the form of overt disease after puberty. This fact, supported by long-range follow-up studies, is implicit in certain classical epidemiological findings. It has long been recognized that the prevalence of clinical tuberculosis for any given generation is determined during the early years of life. In other words, the mortality rates from tuberculosis can be accounted for by the assumption that every generation has the same pattern of mortality at various ages. Its level of mortality is determined very early in life and remains peculiar to it thereafter. Like all statements based on statistical analysis of occurrence of disease in different age groups, this epidemiological law is valid only for a narrow range of conditions and for a certain time in history. Although the law will need to be amended to fit the circumstances of today, it epitomizes the view, nevertheless, that one of the most effective approaches to the control of tuberculosis is to attack the disease among the young.

The only certain way to prevent childhood tuberculosis is, of course, to prevent exposure to tubercle bacilli. This aspect of the problem is not on our agenda and I shall therefore turn immediately to a matter of more direct relevance to the present program, namely, what can be done to minimize the chances that exposure to bacilli will result in the establishment of infection and in the development of disease. Many different and independent approaches to this goal can be envisaged. I shall point briefly to a few, merely as an introduction to the more competent and thorough discussions that we shall be privileged to hear.

To begin with, one could inquire whether it is not possible to base programs of control on the fact that children of school age infrequently develop clinical disease before puberty although they readily contract infection. This profound difference in the response to the presence of tubercle bacilli in the tissues, depending upon the age group to which the infected individual belongs, points to the role of physiological factors in conditioning the severity of the disease. Would it not be worth while, therefore, to attempt to identify these factors, and to control at will their operation in the different ages of life? Alluring as this prospect is, there seems to be little chance, unfortunately, that it will be converted into reality in the near future. For there are today but generalities, and unproved hypotheses, to account for the physiological basis of susceptibility and resistance.

We are on more secure ground when considering the increase in resistance brought about by immunological mechanisms. There is no doubt that one can increase the resistance of man to tuberculosis by vaccinating either with living cultures such as BCG or the vole strain, or with bacillary suspensions killed by a variety of techniques. Indeed it is my personal conviction that immunization can be achieved by vaccinating with purified soluble extracts of the bacilli. However, the very fact that opinion is still divided concerning the usefulness of antituberculous vaccination points to the existence of variables as yet ill defined. In my opinion, the controversies on this subject originate from the variety of ecological circumstances to which any vaccination program must be fitted. The real question is not whether it is possible to increase resistance by vaccination, but rather whether it is worth while to vaccinate at all? And the correct answer may well be yes or no, depending upon environmental factors, many of which are rapidly changing with the epidemiological situation.

Although the modalities of use and the results of vaccination will be discussed this afternoon by scholars experienced in this field, I shall make bold to bring up two theoretical problems which bid fair to play a role in future policies of public health. It is certain, I believe, that the vaccinated individual possesses increased resistance to tuberculous disease. But experiments in animals and observations in man make it likely that vaccination often fails in preventing the lodgment and the persistence of virulent bacilli in the tissues. Vaccination merely retards bacillary proliferation. Thus, the long-range effectiveness of vaccination may depend ultimately upon the operation of the factors which control the fate of pathogenic organisms in a state of latency in the tissues; and these factors in turn are an expression of the total environment in which the vaccinated individual has to live. Analysis of this problem is rendered the more difficult by the

lack of any immunological technique of proved value for the measurement of antituberculous immunity or of activity of the disease process. It may not be amiss in this connection to examine briefly the peculiar relation that skin tuberculin allergy bears to these problems.

There is no evidence to date that the state of allergy to tuberculin is related to the level of resistance; yet the tuberculin test is used almost universally as a measure of the success of vaccination. There is no doubt, on the other hand, that the tuberculin test is the most specific and sensitive indicator of infection available at the present time; and, unfortunately, vaccination deprives the physician of this essential diagnostic tool by rendering the vaccinated individual tuberculin positive. These facts, well known to all, are not mentioned here as arguments against vaccination but rather to emphasize the need for a new approach to the immunochemical analysis of tuberculous infection. Dr. Raffel will discuss these important problems tomorrow. May I be permitted to trespass on his field and express my personal conviction that it will prove possible in the future to prepare, on the one hand, a protective antigen capable of eliciting immunity without tuberculin allergy; and to discover, on the other hand, new immunological reactions specific for tuberculosis yet independent of the tuberculin test. Unfortunately, these immunological problems are not fashionable at present. Little progress has been made in their study, and the faith that they can and will be solved in the future is of little help in dealing with the practical problems of today. Nevertheless, it is wise to remember that their solution would be of practical importance, not only for the development of a new vaccine, but more importantly, perhaps, because it would offer the hope of new diagnostic procedures more convenient than the tuberculin test.

The regrettable neglect of immunochemical studies can be traced, of course, to the spectacular achievements of chemotherapy during the past decade. These achievements have fostered the hope that the generalized use of drugs and of adjunct therapeutic procedures will soon bring about the eradication of tuberculosis. To justify their hope, the optimists can point to the fact that the chemotherapy of tuberculosis is after all only ten years old; they see no reason to doubt, therefore, that eradication of the disease by chemotherapy can be anticipated since new types of drugs will certainly be discovered and more rational procedures developed for their use. For the time being, however, it must be admitted that the beneficial effects of chemotherapy still fall short of the ultimate goal. For this reason, it seems more useful to discuss the limitations of our knowledge in this field than to emphasize the wonderful results that have already been obtained.

Among the unsolved problems of tuberculosis, I shall single out for discussion the fact that under practical conditions the very methods of treatment which are usually so successful in arresting the disease rarely succeed in completely eliminating the bacilli from necrotic areas. This failure has large implications in many phases of the management of tuberculosis in childhood. There are on record, for example, a few cases in which it has been possible in guinea pigs and in man to reverse tuberculin allergy by instituting drug treatment very soon after

infection. More commonly, however, it is found difficult, if not impossible, to change the allergic state once the infection has proceeded far enough to produce necrosis. If we regard the persistence of tuberculin allergy as evidence that tubercle bacilli are still present in the tissues, then it follows that the chemotherapy of primary infection can become eradicative only if linked to a program permitting very early diagnosis. In this country, at least, few are the communities where tuberculin testing at short intervals of time is organized in a manner systematic enough to permit the recognition of tuberculous infection in its initial phase.

Another aspect of the problem has been widely publicized during recent months. From the results of limited experiments in guinea pigs, it has been concluded that treatment of primary infection with isoniazid might be used as a method of immunization more effective than vaccination with BCG. No new experiment was needed, of course, to establish that tuberculous infection elicits in the infected individual a state of heightened resistance to superinfection. The real problem is to demonstrate that immunity thus acquired is worth having. In my opinion, the knowledge required to reach a decision on this point is not yet available.

I am not questioning here the advisability of the use of chemoprophylaxis in primary infection. Indeed, there is sufficient evidence in favor of the protective effect of antimicrobial drugs against the danger of later hematogenous and pulmonary consequences of primary infection to make imperative a study of this problem in its own right. But chemoprophylaxis is not immunity and I do question the relevance of the published information to the problem of antituberculous immunity.

Consider, for example, the two following alternatives. If, on the one hand, chemotherapy has been applied very early after infection and continued long enough to bring about the destruction of all of the bacilli, can we be sure that the immunity induced by the primary infection will be sufficiently lasting to be useful? Is it not likely that a level of immunity high enough to protect against superinfection can be achieved only by permitting the bacterial population to synthesize *in vivo* an adequate amount of antigenic material. On the other hand, if chemotherapy does not succeed in eliminating all the living bacilli, then the residual infection remains a threat to the treated individual in the future. His infection can relapse, just as infection is known to relapse in so many young adults who have overcome their disease, either naturally or as a result of therapy. These alternative situations have been well studied in the case of malaria, syphilis, and scrub typhus. If the experience gained from these diseases can be generalized and applied to tuberculosis, infection-immunity can be achieved only by allowing the disease process to reach a sufficient stage of development before arresting it by drug treatment. The question then arises again: Is the benefit to be derived from infection-immunity worth the risk attendant on the presence of bacilli in the tissues? Many subtle aspects of the ecological situation need be considered before one can answer this question in a manner meaningful for the control of tuberculous infection as it occurs in man under natural circumstances.

It is clear from the trend of scientific publications that these problems are being carefully considered in many quarters. Since they will certainly be part of your deliberations, it may be wise to emphasize at the outset that certain theoretical uncertainties render difficult the interpretation of experimental and clinical findings, especially of those based on the tuberculin test. As already mentioned with regard to vaccination, there is no evidence that tuberculin allergy can be used as a measure of immunity. Furthermore, it is not known whether persistence of a positive tuberculin test means that living bacilli are still present in the tissues. Indeed, it has been repeatedly shown that a lasting and intense allergy can be caused in man and animals by the injection of small amounts of killed bacilli. It is not at all sure, on the other hand, that the reversal of the tuberculin test necessarily means that all living bacilli have been eliminated from the tissues. In fact, all available evidence indicates that it takes a fair amount of bacillary material to elicit and maintain the allergic state; whereas, in contrast, a few surviving bacilli are sufficient to reactivate infection. Thus it is not possible by the use of the techniques presently available to determine with certainty the effect of therapeutic procedures on the infectious state of the patient. Progress in this area of pathogenesis will require greater knowledge than is presently available, not only of the properties of tubercle bacilli, but even more, I believe, of the structure and evolution of the lesions in which the living bacilli find shelter and in which are stored the products of bacillary disintegration. For this reason, it was very wise, in my judgment, to place early in the program a discussion of the histopathological characteristics of tuberculosis.

Granted that there are still many unknowns of great theoretical importance, it is certain nevertheless that recent practical developments make possible—and indeed compel—a reformulation of the policies for the control of childhood tuberculosis. The prospectives are alluring, but prospective without retrospective can lead to a false sense of values. We are very fortunate, therefore, in having on our program this morning two investigators who have been identified with many different phases of tuberculosis for many years and who have given much thought to the evolution of the disease. I wish to thank the organizers of this Symposium for having given me the honor to preside at the meeting devoted to the presentations by Drs. Kornel L. Terplan and Georges Canetti.

# MORPHOLOGIC ANALYSIS OF FATAL TUBERCULOSIS IN CHILDREN

## With Some Comments on Endogenous Exacerbation and Tuberculous Meningitis

KORNEL L. TERPLAN[1]

I should like to express the thought that much of the material which I have prepared for this conference is, today, in the modern era of tuberculosis treatment, of largely historic interest. To some of you, it might sound, perhaps, somewhat anachronistic to present this rather dark background for discussions on successful treatment and prevention of the disease. I prefer, however, to analyze only post-mortem material from children who have died from tuberculosis during the past fifteen years in a General Hospital for Sick Children, with but few additions from a tuberculosis sanatorium. The major part of it was observed before the advent of the antimicrobials. My experience with surgically removed tuberculous lesions or entire lobes from children is very limited.

The principal task of a pathologist is to attempt the clarification of fatal diseases in man. Tuberculosis in children, although markedly reduced in numbers of infected and diseased, is still a fatal disease and nearly one-fourth of this material is from the past eight years.

But first, at this rare and festive occasion of an international symposium on tuberculosis in infancy and childhood, let me pay homage to those investigators to whom we owe most, if not all, of the fundamental morphologic facts regarding primary tuberculous infection: to the French school of Parrot, his famous law of the *lymphadenopathie similaire*, which preceded the discovery of the tubercle bacillus; to his pupils Hervuet and Hutinel; to George Kuss, Monrad, Naegeli, Eugen Albrecht, and to the Austrian school: Heinrich Albrecht and, in particular, Anton Ghon. I wish that all who are still engaged in morphologic and pathogenetic studies of tuberculosis would be guided by the thoroughness of anatomic analysis as revealed in the classic papers of Parrot, Kuss, and Ghon.

These fundamental anatomic studies, based originally on fatal tuberculosis in children, have been applied to much laborious work in trying to analyze the more common disease, chronic pulmonary tuberculosis beyond the age of childhood. The law of Parrot and whatever we have learned since about the intrabronchially spreading primary infection still form the only rational basis for any pathogenetic analysis of chronic pulmonary tuberculosis, particularly in the adult.

Fortunately for the pathologist, the problem of true reinfection from without rarely, if ever, arises in the course of tuberculous disease in the child, even if it might have extended into adolescence. Post mortem, the more or less direct linkage of the various changes to the primary lesion usually appear quite obvious.

[1] Buffalo Children's Hospital and the University of Buffalo School of Medicine, Buffalo, New York.

I would like to quote a few lines, written in small print, from one of the classic papers on tuberculosis in children, by Ghon and Roman:

> On the basis of anatomic considerations, we should expect all transitional types between cases with typical progressive pulmonary tuberculosis in the presence of an old primary complex and cases of phthisis in which a complex is missing, and the progressive tuberculosis, developed directly from the primary lesion.

Little, if anything, can be added to our pathogenetic understanding of pulmonary tuberculosis in general.

The evolution of the progressive primary infection in children is well known, as well as the less continuously developing forms based on clinically latent infections. For many months or several years, they might completely escape detection until one day, for reasons not altogether clear, the dreaded picture of tuberculous meningitis is noticed.

It might be largely the result of chance that, in the cases to be presented, the first symptoms of the tuberculous infection, with but few exceptions, were those of oncoming meningitis.

This survey is a continuation of the studies on children which were reported nearly fifteen years ago. As to the method of study, it proved most helpful, particularly in the age group beyond infancy, to precede the anatomic examination by post-mortem roentgenography. Such pictures are, in addition, of good teaching value for students. This method facilitates the search for shadow-giving lesions, particularly in the state of chalky or "stoney" regression. It frequently demonstrates the extent of the primary complex as well as good dissection. Its importance, however, will be realized particularly by those whose interest is also directed to abortive infections, restricted to very small lesions which otherwise, as you will see, probably would escape detection. A few words on this facet of our problem in relation to the tuberculin test will be added later.

The incidence of fatal tuberculosis in children during the past fifteen years is shown in table 1, taken from a total figure of 2,500 deaths, controlled post mortem; of these, 1,088 ranged between three months and fifteen years of age. In line with numerous experiences elsewhere, most of the patients died during the first two years of life, very few being less than six months of age. While the percentage of 4.6, excluding all deaths of children less than three months old, ap-

TABLE 1

INCIDENCE OF FATAL TUBERCULOSIS IN INFANCY AND CHILDHOOD
1940–1954

| | |
|---|---|
| Total number of post-mortem cases | 1,088 (3 months to 15 years) |
| Tuberculosis as cause of death | 50 (4.6%) |
| From the following age groups: | |
| 3 months to 5 months | 2 |
| 6 months to 24 months | 27 |
| 25 months to 4 years | 14 |
| 5 years to 15 years | 7 |
| Total | 50 |

TABLE 2

PATHOGENETIC TYPES OF FATAL TUBERCULOSIS

| | |
|---|---|
| Primary cavities | 8 (2 Negro patients) |
| Caseated pneumonia | 5 (2 Negro patients) |
| Primary complex with dense overwhelming miliary tuberculosis or more scattered hematogenous tubercles | 25 (5 Negro patients; 1 Indian) |
| Protracted progression (from endogenous exacerbation) | 12 (2 Negro patients) |
| Origin of exacerbation: | |
| Chalky or calcified primary pulmonary complex | 7 |
| Tuberculous ostitis and/or caseated chalky genital organs | 2 |
| Calcified intestinal lymph node | 2 |
| Calcified, chalky adrenal | 1 |

pears rather high, a breakdown of this figure relative to the succeeding calendar year groups shows a reduction from 2.5 per cent before 1945 to 0.5 per cent for the past two years. No death has been observed by us since May, 1954.

The anatomic types of these 50 fatal cases are listed in table 2.

Distinct cavitation of the primary focus and massive caseated primary pneumonia were observed largely in the young child less than two years of age. The percentage of Negro children in this group is greater than in the others. Half of all of our cases showed the classic picture of a progressive primary complex with lymphohematogenous tuberculosis terminating in tuberculous meningitis. Our interest was centered more on the last group in the table, with findings pointing to a more protracted evolution of the disease and, in some instances, to its exacerbation from older extrapulmonary lesions. The endogenous sources for the fatal tuberculous meningitis are also listed in table 2. In 7 of the patients, the usual histologic routine check for miliary tubercles in such organs as spleen, liver, and kidney failed to disclose any hematogenous tuberculosis; nor was there any gross evidence of miliary tubercles outside of the central nervous system. In the other 5 patients there were extremely few tubercles in the liver and in the spleen.

The apparently selective involvement of the meninges in all of these cases naturally poses a most difficult problem. In particular, in none of these cases was there any original encephalitic tubercle to which the massive tuberculous meningitis could have been traced. If, for instance, a florid caseating tuberculosis of the adrenal in connection with a hematogenous calcified tubercle can be proved, encroaching upon the lumen of the medullary vein with numerous acid-fast bacilli, it is strange that we should fail to find hematogenous tubercles in the lung or in the spleen and only a very rare one in the liver.

If the obvious source of the exacerbation is in the chalky, calcified complex of a primary pulmonary complex, with acid-fast bacilli readily seen between the calcified particles in the center of a lymph node, it is again hard to realize that no other hematogenous tubercles are found, but only the typical picture of diffuse tuberculous meningitis. Recent tuberculous changes in these cases of so-called lymphogenous endogenous exacerbation (Ghon) do not always extend into the angulus lymph nodes. Such recent tubercles, caseated or cellular, were

present in some; absent in others. The role of broad mediastinal adhesions about the extensive chalky tuberculous lymph nodes, particularly in the right upper tracheobronchial and paratracheal group, in relation to venous anastomoses to the vertebral bodies or to the epidural and meningeal veins is not known. There are a few observations of recent subapical tuberculosis of the young adult, with localized mediastinal adhesions and selective tuberculoma formation of the brain or with tuberculous meningitis, but without any other finding of a blood-borne spread.

A series of photographs[2] shows the different original pulmonary changes and, in some, the probable sources for the tuberculous meningitis.

First, the typical pictures of the progressive primary lesions in the lung:

1. Typical primary complex
2. Massive primary tension cavity, with minimal lymph node reaction—death from phthisis
3. Progressive primary tuberculosis with a perforating lymph node and combined intrabronchial and hematogenous seeding (present in 4 cases of this series)
4. Massive caseated pneumonia with cavitation and miliary tuberculosis
5. Massive miliary tuberculosis from an extensive chalky complex
6. Protracted progression (590–161)
7. [1] *1212* — *1594* — *1257*
   2½ years — 18 months — 2 years 8 months
   numerous tubercles — scattered hematogenous tubercles
   *1209* — *2018* — *2056* — *637*
   15 years — 11 years — 8 years — 7 years

I should interject here a few words about incidental findings of tuberculous changes. Only cases with post-mortem roentgenographic control have been analyzed. Histoplasma infection in children in our area is thus far negligible. Restricted tuberculous infection, with a typical "closed" primary complex, or with merely one or more small parenchymatous primary tubercles, were less frequently seen in the past ten years than in the preceding fifteen years. In some of these, abortive foci without lymph node changes, acid-fast bacilli were found in very small numbers in the decalcified chalky or "stoney" center of the lesion. No trace, however, could be found of any tuberculous lesion in the lymph nodes regional to such foci. In these cases, no information on the tuberculin test was available. In another instance, one minute calcified primary tubercle was present in the lung and a few acid-fast bacilli were present in one bronchopulmonary lymph node. Here the positive result of the tuberculin reaction, several weeks before death, was known.

It would be of great interest to have collateral information on the tuberculin reaction in all of these cases with minimal parenchymal tubercles, particularly if we still can demonstrate acid-fast bacilli in them.

[1] Not available for publication—*The Editor.*

[2] In the near future, it is intended to submit for publication in the *American Review of Tuberculosis and Pulmonary Diseases* the detailed information and some of the photographs of these cases.

One is reminded of the much greater interest of some pathologists in this question half a century ago, when the systematic tuberculin testing of the Viennese children in the Pediatric Clinic of Hamburger had acted as a great stimulus to Ghon and his pupils. They analyzed most meticulously, post mortem, the organs of children who had died from causes other than tuberculosis. At first they did not find any tuberculous lesions. But, relying on the meaning of a positive tuberculin reaction in an infant or young child, they discovered—after considerable search—the very early exudative lesions teeming with bacilli.

The analysis of the table on tuberculous meningitis reveals two infants, three and four months of age, of particular interest. In one, only by careful gross and histologic examination of the entire brain were several small recent meningeal tubercles found; but none were found in the brain substance. In the other, several recent meningeal tubercles and one small tubercle in the medulla were discovered. In neither of these cases was there any diffuse tuberculous meningitis. The basal cisternae were not involved. It is well known that tuberculous meningitis is rarely observed before the age of four months.

One or more tuberculomas in the presence of typical tuberculous meningitis were seen only in those cases of our series in which dense miliary tuberculosis had occurred. One of these is of particular interest because the meningitis had escaped the basal cisternae. It was clearly seen around a large tuberculoma in the cerebral cortex, with tubercles extending from there toward the Sylvian fissures. There were several tuberculomas in the cerebellum, in addition to a few meningeal tuberculomas on the surface. Or again, in another case of this series, there were multiple tuberculomas in the dura, in the leptomeninx, and in the brain. The source for the diffuse tuberculous meningitis, which was present in this case, could not be traced to a single lesion.

Opinions on the pathogenesis of the acute diffuse tuberculous meningitis still differ considerably, depending to some extent, perhaps, on the source of the material and not necessarily on the thoroughness of the analysis. Meningeal tubercles, formed particularly within sulci, are apt to develop into small meningocortical foci.

In addition, one or more tuberculomas, in various parts of the brain, pons, cerebellum, or frontal lobe, can occur, leading to distinct localized meningeal tuberculosis (sometimes with tuberculous adhesions to the dura) without, however, causing diffuse tuberculous meningitis. Only tuberculomas close to the surface of the ventricular system, as has been clearly demonstrated by various investigators, may lead to massive infection of the circulating cerebrospinal fluid in a fashion similar to purulent meningitis, which always occurs when an abscess ruptures into the ventricular system.

For an evaluation of the hoped-for results of modern treatment, the early diffuse changes in tuberculous meningitis should be clearly understood (whatever the source for massive infection of the circulating cerebrospinal fluid may be) to be due to one or more meningeal tubercles or small subpial cortical tuberculomas. Only the small incipient tubercles on the surface or in the substance of the brain will be readily reached by the antimicrobials. Once larger nodules are

formed, with the characteristic picture of the proliferative tuberculoma, the center seems to retain the necrotic lesion, in which tubercle bacilli, although small in number, could be demonstrated in all the treated cases which I have analyzed thus far.

In the acute state of tuberculous meningitis, the subarachnoid spaces are filled with exudate rich in fibrin, and inflammatory cells, leukocytes and monocytes. The small arteries and arterioles are very severely affected in a fashion characteristic of allergic inflammation with marked fibrinoid necrosis. The collateral superficial cortical inflammation is already quite impressive in these early phases. Sometimes, rather early, considerable necrosis can be seen in the fibrinous exudate, flooded with leukocytes and mononuclear cells. The veins show massive infiltrations in their walls, but no necrosis. Occasionally thrombi are formed in the veins, causing hemorrhages in the cortical areas which they drain. Only when caseation becomes more prominent does one recognize the formation of cellular tubercles in this still recent state of meningitis.

The absence of distinct tubercles and the presence of a nearly phlegmonous and largely nonspecific exudate with necrosis characterize the picture of recent diffuse tuberculous meningitis. Even if the walls of the blood vessels are massively inflamed, complete occlusion of the smaller arteries by endarteritic proliferation is not seen as frequently as in the chronic cases. However, conspicuous swelling of the arteries, particularly of the intima, from edema in the allergic state of recent polyarteritis is sometimes very marked. Within the sulci, the beginning formation of meningocortical tubercles can already be seen in this early phase. The acute changes affecting the arteries and veins on the surface of the brain, and particularly in the sulci, are apt to produce infarct-like liquefaction. These we had seen in almost every case before the chemotherapeutic era.

In the treated case, the picture is entirely different. It is, in particular, the almost tumor-like nodular enlargement of the meningeal and meningocortical tubercles which is the most characteristic distinction from the nontreated case. The smaller meningeal arteries in the leptomeninx show very marked endarteritic protrusions, "pillow" formations, sometimes almost completely occluding the lumen. Extensive infarctions have been observed, particularly in the area underneath these huge meningocortical tuberculomas, including the base of the brain stem, where these tumor-like nodules are completely filling out the cisternae. In all of these cases very marked hydrocephalus developed, even if smaller tubercles on the surface seemed eventually to be replaced by fibrous tissue. In fact, the formation of meningeal tuberculomas in the treated case is so marked that it resembles gliomatous tumors of the cerebellum which frequently extend through the foramina to the base of the brain stem. Neurologic symptoms in such cases might be very similar to those of a medulloblastoma in the child. For example, we have encountered a patient who had distinct meningitic symptoms and a positive tuberculin test, and was treated with streptomycin. At post-mortem examination a completely calcified primary pulmonary complex was found, as was a meningeal extension of a medulloblastoma with tumor-like protrusions, just as is seen in meningeal tuberculomas of the treated cases of tuberculous meningitis.

# DYNAMIC ASPECTS OF THE PATHOLOGY AND BACTERIOLOGY OF TUBERCULOUS LESIONS

GEORGES CANETTI[1]

It is fascinating to study tuberculous lesions from a viewpoint in which morphology and biology are integrated. Numerous biologic problems are in fact suggested by each morphologic feature. In any disease, the structures seen under the microscope are nothing more than a sort of crystallization of the major biologic problems, those raised by host-parasite relationships.

I shall not try to describe *all* of the varieties of tuberculous lesions; it is necessary to make a selection. We can make a classification and divide tuberculous lesions into two main groups: those in which the bacilli are multiplying, and those in which the bacilli are destroyed. Since we have to choose, we had better choose the more agreeable lesions in which the bacilli are destroyed. Consequently, the problems dealt with here will to a large extent be in the field of immunity and resistance.

In man there are two main circumstances in which tubercle bacilli are destroyed. They can be destroyed in the solid caseous lesions, and they can be destroyed in macrophages. We shall examine these two circumstances in turn. In conclusion, we shall study the way in which the considerable changes, brought about by present-day prophylaxis and therapy, could, in the future, change the relative importance of these two mechanisms in the evolution of tuberculosis.

*Death of bacilli in solid caseous material:* When man becomes infected by tubercle bacilli, he develops a double lesion which is both a pulmonary and a lymph node lesion and which is known as the *primary complex*. Generally, the scarred remnants of these lesions persist indefinitely and are evidence of a tuberculous infection which has been overcome. This double lesion is always caseous at some time; the phenomena of calcification and ossification, which characterize its healing, always take place in a central caseous core, surrounded by a more or less thick capsule of fibrous tissue. When this central core has reached the stage of calcification, *it no longer contains viable bacilli in more than 80 per cent of cases* (1, 2). This has been shown by numerous investigations. The normal progress of the stable necrotic lesion thus tends towards sterilization. Before becoming caseous, the lesion was *exudative*. When examined at the stage of exudation, the lesion is rich in macrophages. These cells constitute its principal component. In the macrophages there are many bacilli. Their number is still further increased in the following stage, in which occur the destruction of the macrophages and the beginning of general necrosis. It is only when the necrosis tends to become homogeneous that the number of bacilli diminishes. This is the first step of the regressive process which is ultimately conducive to sterility in the lesion.

Histologic and bacteriologic study of the primary complex thus shows that it is not during the macrophagic stage that the deciding factor for biologic cure of the lesion is produced, but during the stage of necrosis. The stage of phagocytosis

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is inoperative. It is only in the new medium formed by necrosis that the factor or group of factors which are responsible for the death of the bacilli appear. This factor is not "immunologic" in the usual sense of the word, since classical immunology only deals with the cellular factors and antibodies which play an active part in the fight against bacteria. But, if one includes in the term "immunity" *the total of all organic phenomena* which oppose the extension of an infective process (which is reasonable in view of the etymology of the word), then the death of bacilli in the solid caseous material is certainly an immunologic phenomenon.

What we have just said concerns the *latent* tuberculous infection, which even today affects the majority of people at some stage of their lives. But the phenomenon of the death of the bacilli in the solid caseous material occurs to the same extent in the course of *active tuberculous disease*—in pulmonary tuberculosis, for example.

In pulmonary tuberculosis one almost always finds a greater or smaller number of *solid* caseous nodules in addition to the principal lesion, which is generally a cavity. It is very rare to find a case, however mild, in which these accessory lesions are not present. Some time ago, while studying the bacillary content of tuberculous lesions removed by resection, I decided not to limit myself to examining the principal lesion, but also to culture one or two solid nodules wherever I could find such caseous nodules.

Solid caseous nodules which were surrounded by a macroscopically visible capsule, that is to say, which had already reached an advanced stage of cicatrization, were obtained from 212 patients. Culture for tubercle bacilli was negative in 74 per cent of the nodules. Many authors have made similar observations, particularly Auerbach and Hobby and their collaborators recently (3). Tuberculous nodules are small lesions, having an average size of 3 to 8 mm.; one can culture them as a whole. The small percentage of positive results is, therefore, not explained by the fact that only a part of the lesion may have been studied. In these lesions the death of the bacilli has occurred just as it has in the primary focus of latent tuberculous infection; in both cases the phenomenon is the same.

It is necessary to mention one important clarification concerning previous chemotherapy undergone by the patients. Provided that the state of the nodules is really that which has been described, i.e., with macroscopic encapsulation, *the frequency with which tubercle bacilli can be cultured is the same whether chemotherapy was prolonged or short, or never given at all.*

In cases in which there has been chemotherapy, one cannot say that it has not, partially or completely, caused the death of the bacilli; but, one can say that the phenomenon occurs perfectly well without chemotherapy. It is, therefore, a quite general occurrence, and one must consider it to be one of the major immunologic events in tuberculosis.

I would like to make a digression here into the problem of the visible, but not viable, bacilli which exist in many necrotic tuberculous lesions. This is a problem which has a remarkable gift for arousing great passions and I am not going to take the risk of dealing with that aspect of the problem here. In the controversy

which has arisen about the visible but not viable bacilli, there are two sides. Certain authors believe that there is a great probability that these bacilli are simply dead; other authors maintain that there are good reasons for thinking that these bacilli are alive (although in a state of extremely reduced metabolism), and that it is simply that our present methods of culture are inadequate.

In any case, the existence of this controversy and the proportions to which it has grown have led many authors to the following concept: what happens in the solid caseous lesion is simply a massive transformation of viable bacilli to nonviable bacilli. Whatever the exact significance of their nonviability, the bacilli initially present are always there. It is about this idea, which is to a certain extent instinctive, that I would like to say a word, since it does not correspond at all to the true situation.

What happens in the solid caseous material is a *disappearance* of the great majority and very often of all of the bacilli, and not merely a change in their viability. I have studied this phenomenon at length, and it has been seen, moreover, by numerous investigators. Quite recently, Wayne and Salkin (4) described it with great accuracy. They have shown that the ratio of visible bacilli/viable bacilli, which appears to be very high in lesions resected shortly after the sputum becomes negative for tubercle bacilli, tends towards unity in certain lesions resected several months after this conversion. The great majority of the visible bacilli have, therefore, certainly disappeared from these lesions. The problem of the visible, but not viable, bacilli (the importance of which should certainly not be underestimated) does not, therefore, affect the major phenomenon of the death of the great majority, and sometimes of all, of the bacilli in the solid caseous material. These are just two different problems.

This is not the place to discuss the causes of this phenomenon at length. Everybody knows the work done by Dubos and Hirsch (5–7) on the bacteriostatic or bactericidal action on the tubercle bacillus of certain substances (fatty acids of the aliphatic series, spermine, spermidine) assumed to be present in inflammatory and necrotic areas. The most significant of these experiments, for the problem which interests us, seem to be those by which Dubos (6) has shown that the bacteriostatic action of certain substances, for example, lactic acid or alanine, is converted to a bactericidal action in a growth medium under anaerobiosis. For, anaerobiosis is the condition to which the bacilli are subjected, to a greater or less extent, in the solid caseous material. Similarly the existence of both anaerobiosis and an accumulation of carbon dioxide together, has, according to Dubos, a more pronounced bactericidal action than each of these factors taken singly; this condition also might exist in the solid caseous material. I believe that research of this nature is of great importance. However, there is great need of more intensive study of the chemical composition of caseous material. The question whether the bacteriostatic and bactericidal substances assumed to be at work in necrosis actually exist there in active concentrations remains to be settled. The practice of surgical excision nowadays provides us with unhoped-for amounts of caseous material. Biochemists working on tuberculosis should remember this.

*Death of bacilli in the macrophages:* Although this paper began by a study of

the death of bacilli in the solid caseous lesion, this was not meant to suggest that the bacilli cannot succumb in man as a result of the action of macrophages. There is one particularly eloquent fact among those which demonstrate that this factor can be perfectly effective; that is, the fate of the bacilli which are spread in persons who have pulmonary cavities.

Let us imagine a tuberculous patient of fifteen years ago who has a large cavity. Chemotherapy does not exist. The patient is coughing up sputum containing many bacilli ten times or more a day. Consequently, bacilli are present in the bronchi of this patient practically all of the time.

These are ideal conditions for the appearance of new caseous lesions. No exposure to outside infection, however severe, can even approach infective conditions of such severity. Nevertheless, the rate at which metastatic caseous lesions appear in such a subject is very slow. Many months can pass without alterations in the roentgenographic picture.

Should we assume that the bacilli found in the bronchi of such a patient are not being aspirated into healthy pulmonary sites? This is most unlikely, because these patients make particularly large respiratory movements while coughing. These movements must lead to the dispersion of the bacilli.

We are, therefore, faced with the following conclusion: If bacilli from the cavity frequently reach healthy pulmonary regions, and if they only rarely produce new caseous foci (compared to what they might do), it is because the phagocytic defense is extremely efficient. By inhibiting the multiplication of bacilli, the phagocytic defenses prevent the development of the number of bacilli necessary for the production of a new caseous lesion. The problem of knowing whether the macrophages are helped in this action by antibodies has been, and will continue to be, much discussed. Until now, the experimental evidence does not favor this hypothesis at all; nevertheless, it has its supporters.

Thus, the phagocytic defense mechanism can be remarkably effective in man, as shown by the very slow course of cavitary tuberculosis even in the absence of chemotherapy. Nevertheless, this level of efficiency is not permanently maintained. In the untreated patient with a cavity a moment *always* comes when new caseous foci will appear. It is only a question of time.

What, then, causes the failure in the activity of the macrophage which one day makes this deterioration possible? This is a very important problem, and it is as yet entirely unsolved. One can only put forward several hypotheses.

It may be that the decisive factor is the *number of bacilli* which the macrophages have to confront. It is conceivable that, if a macrophage happens to be confronted by a clump of bacilli (clumps of such bacilli occur in the walls of cavities and can be dispersed as such), it will be destroyed by the ingestion of the clump of bacilli, just as an *in vitro* culture of macrophages succumbs if too heavily infected.

It could be that one is dealing rather with a genetic resistance put up by certain bacilli against the macrophages. In the same way as bacterial mutants occur which are resistant to chemical agents and antimicrobials, there may

exist bacterial mutants resistant to the antibacillary factors which are operating in the interior of the macrophages.

Finally, it could be that the whole matter is a question of the hormonal balance existing at the moment when the bacillary spread occurs. Lurie (8) has performed experiments on rabbits infected by the human bacillus and treated with cortisone. These experiments have shown that cortisone inhibits the destructive action of the rabbits' macrophages on the human bacillus. This action is independent of the well-known anti-inflammatory action displayed by cortisone, the effects of which are shown equally well in his experiments.

This experimental fact is of prime importance. Perhaps it provides a clue to the mystery of the antibacillary activity of macrophages being sometimes effective and sometimes ineffective, a major problem in the natural history of tuberculous infection.

What happens, from a morphologic point of view, when the phagocytic defenses have been effective and have finished up by destroying the bacilli? There appears a lesion of the tubercle type. This lesion is known to consist of epithelioid cells, giant cells, and lymphocytes in variable proportions. The evidence that the tubercle is related to the destruction of the bacilli is provided experimentally by the work of Lurie.

Lurie has shown that, in experimental tuberculosis of the rabbit, whenever there is a reduction in the number of bacilli after an initial phase of multiplication, the macrophages of the lesion at the site where the reduction occurs are transformed into epithelioid cells (9). In previously vaccinated animals, when the destruction of bacilli begins earlier, there is also an earlier conversion of macrophages into epithelioid cells (10). There is a close relationship between the two phenomena. Taken together with the usual absence of tubercle bacilli from human tubercles (only rarely are bacilli found in epithelioid cells and even in giant cells), this constitutes proof that the tubercle is closely related to the destruction of bacilli (1).

But the actual mechanism by which macrophages are converted to epithelioid and giant cells is still uncertain. The earliest explanation was that this was a *chemical* process, that is to say, that there are certain bacillary components which, when liberated by destruction of the bacilli, produce epithelioid metaplasia of the macrophages.

But this hypothesis, which has given rise to some remarkable work, especially that of Sabin, Doan, and Forkner (11), continues (in spite of some progress) to run up against the large doses of bacillary constituents which must be injected into the animal to reproduce the cellular metaplasias. Such doses could not possibly exist in tuberculous infections.

Recent work points toward a different mechanism. Epithelioid metaplasia may arise as the result of an antigen-antibody reaction taking place in macrophages; but I will not go into a discussion of this problem.

The tubercle presents no major problems from the point of view of prognosis. Although necrosis of the tubercle may occur, it generally heals by involution of

the metaplastic cells and sclerosis. The tubercle usually indicates that the immunologic response of the organism has been adequate.

* * *

Before beginning the last part of this paper, we must summarize the facts which have been elaborated. In man there are two important means of destroying tubercle bacilli—the macrophages and the solid caseous material. We could, if we liked, express matters in military terms, and say that these two mechanisms are arranged like two battalions of troops in a defensive system. The defense put up by the macrophages enters the lines first. If it succeeds, the bacilli are destroyed and there is the formation of a tubercle. If it fails, the bacilli multiply and there is caseation.

The second defensive battalion, composed of the intracaseous antibacillary factors, now enters the lines. There are two possible results. Either there is a victory of these factors, and the bacilli are destroyed in the solid caseous material. Even if they are not *all* destroyed, the few remaining bacilli will not begin to multiply again. This is the most frequent result; and this is what regularly occurs in latent tuberculous infection.

Alternatively, there is a setback. The bacilli, which have been only partially destroyed, sooner or later begin to multiply again within the caseous lesion. Simultaneously, the necrosed area softens, and the formation of a cavity occurs. In this way active tuberculous disease supervenes. The bacilli of the cavity are inevitably spread. Against them, at a new site of colonization, the first immunologic mechanism, that of the macrophages, once again joins battle. Usually, as we have already seen, this succeeds in its purpose. If it fails, a new caseous lesion is formed, and this is again the site for the second immunologic mechanism to come into play, and so on.

Thus, the progress of tuberculosis involves a ceaseless alternation of the two immunologic mechanisms. Each has victories to its credit, each suffers defeats, and each tries to salvage the damage which has been suffered through the inefficiency of the other.

I would not like to be accused of teleologic thinking for having summed up things in this manner. What I wanted was merely to describe what happens. There is no experimental reason to suppose that there exists a sort of "high command" ruling immunity.

*Changes brought about by antituberculous chemotherapy:* The last part of this paper consists of a short examination of the following problem: What is the effect of chemotherapy on the relative importance of the two antibacillary mechanisms in human tuberculosis? Toward what morphologic features and toward what new biologic conditions is it likely to lead a tuberculous infection?

This problem must be studied separately for curative chemotherapy and for prophylactic chemotherapy. Curative chemotherapy is treatment applied to tuberculous disease which is already progressing. Prophylactic chemotherapy is applied to prevent or to treat latent tuberculous infection.

Let us first of all examine what happens during curative chemotherapy. Several authors, and Denst (12) in particular, have pointed out an interesting

phenomenon. A remarkably increased number of epithelioid and giant cells can be seen in tuberculous lesions removed by surgical excision after chemotherapy (especially after isoniazid). I, myself, have studied this phenomenon at some length (13, 14); the histologic findings recorded from 174 cases of surgical resection after chemotherapy can be summarized as follows. There is often a notable increase in the importance of the epithelioid and giant cells and lymphocytes in these lungs, compared to what one formerly used to observe in lungs at autopsy. We are not dealing here with an absolute, but with a relative increase in number; it is the *ratio* of the cellular lesions of metaplasia to the exudative or caseous lesions which is greatly altered, the former increasing at the expense of the latter. Furthermore, these cellular metaplasias have more varied structure, especially that of the giant cells. It is much more polymorphic. This fact can be observed whatever the chemotherapy employed: it is perhaps more prevalent after the administration of isoniazid.

The facts elaborated above explain this phenomenon very well. Chemotherapy either inhibits multiplication of the bacilli or destroys them; it, therefore, assists the macrophages. It must necessarily increase the number of foci in which bacillary multiplication does not reach the rates required for caseation. The destruction of these bacilli by the joint action of the macrophages and chemotherapy leads, in accordance with the mechanisms described above, to the appearance of numerous epithelioid cells, giant cells, and lymphocytes. These lesions are, therefore, the precise equivalent of the more numerous caseous lesions which one formerly used to find in the absence of chemotherapy; they are a sort of *histologic trademark* of the intervention of chemotherapy.

Thus, in (active) tuberculous disease, chemotherapy considerably increases the percentage of foci in which the second immunologic mechanism, that of the intracaseous antibacillary factors, need no longer intervene. This change is of very great importance because it is to failure of this mechanism and to its possible consequences, that is to say, to the resumption of bacillary multiplication in the caseous focus, that aggravation of clinical tuberculosis, to a large extent, is due.

The possible effects of *prophylactic chemotherapy* on the immunologic mechanisms are not the same whatever the time at which it intervenes. This weapon can be used against latent tuberculous infection under two different circumstances. Either it is put to work on a subject in whom sensitivity to tuberculin, that is to say, latent infection, already exists; or alternatively, it is given to a subject who is not yet allergic, but is exposed to a strong risk of infection. In the first case, we have in mind prophylaxis of active tuberculous disease; in the second case, prophylaxis of the *latent infection* itself.

We still have no precise information—I mean pathologic confirmation—on the possible effects of these two kinds of chemoprophylaxis in man. Indeed, it would be very difficult to get such information, but experimental evidence gives some support to the following considerations. Chemotherapy, begun before the onset of the latent infection, would probably efficiently oppose any multiplication of the bacilli which penetrated the organism. And, as the number of bacilli which

can, under normal circumstances, pass through the arresting mechanisms of the respiratory tree and penetrate into the alveoli is minimal, there should, in such a case, be neither development of sensitivity to tuberculin nor, of course, appearance of a caseous focus; a tiny lesion of the tubercle type would probably develop. This lesion would be completely healed by sclerosis.

If chemotherapy is only begun after the appearance of the sensitivity to tuberculin, it is very unlikely that it will succeed in preventing the primary lesion from being caseous. We do not quite know the time relationship in man between caseation of the primary complex and the appearance of the sensitivity to tuberculin; but, as the tuberculin sensitivity cannot develop without multiplication of the initially so few inhaled bacilli, there is great likelihood that, by the very fact of this multiplication, the lesion at the implantation of the bacilli (that is, the primary focus) will already have caseated. This is even more likely since, when the presence of allergy is ascertained, it has quite probably already been present for weeks or even months, thus providing ample time for necrosis to occur. Thus, the administration of chemotherapy after the appearance of sensitivity to tuberculin will, in all probability, not change in any way what is the rule in an *untreated* latent infection; that is, the *production of primary caseation*. And since the involvement of the satellite lymph nodes occurs at almost the same time as that of the lung, we would in all probability be dealing as often as before with a *primary caseous complex*.

All the same, these considerations do not in any way rule out the possibility that chemical treatment may produce some useful effects in already established, latent tuberculous infection. It can act with the naturally occurring antibacterial factors which exist in the solid caseous material and, thus, hasten healing of the lesion. Above all, if lymphatic and vascular dispersions of bacilli occur from the still active primary complex, it can stop, at the outset, all attempts of these spreads of bacilli to multiply in distant foci. But, in the main, the primary lesion will still be caseous, that is to say, subjected to the same immunologic mechanism as occurs in the absence of chemotherapy.

* * *

I should like to conclude this paper by a consideration of a more practical nature. Prophylactic chemotherapy of the first type, that is, applied to nonexisting infection in the hope of preventing it, would probably be extremely useful in countries which are medically under-equipped. In such countries, the discovery of a case of open tuberculosis does not necessarily lead to hospitalization of the patient and does not, thereby, abolish the risk of infection of people living in contact with him. Prophylactic chemotherapy under such conditions would make simultaneous vaccination by BCG impossible, or would at least reduce its effects very considerably. We know that the effectiveness of a vaccine is, in large measure, a function of its multiplication in the host, and prophylactic chemotherapy would antagonize the multiplication of BCG as well as that of inhaled virulent bacilli against which it is directed. This necessity for choosing between prophylactic chemotherapy and vaccination has something agonizing about it: because chemotherapy gives protection instantly but cannot be pro-

longed indefinitely; while vaccination gives lasting protection, at least in the majority of cases, but a protection which only starts after an interval of two to four months. Now, I believe that there is a means of avoiding this embarrassing choice, and that is by *creating a BCG vaccine which would be resistant to isoniazid*. The administration of a vaccine such as this would make it possible for chemoprophylaxis and vaccination to be initiated simultaneously. During the time required for development of specific immunity, the vaccinated person would be under the cover of chemoprophylaxis and probably no virulent infection at all would occur. Yet, the establishment of immunity would not be hampered by the administration of isoniazid.

Extensive experimental trials of the effectiveness of a vaccine of this kind would be necessary. But I think that an undertaking of this sort would be of some importance. The prophylaxis of tuberculosis in under-developed countries would probably benefit considerably by the possibility of simultaneous application of vaccination and prophylactic chemotherapy.

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## GENERAL DISCUSSION

DR. JOSEPH DAVIS (Palo Alto, California): I would like to ask Dr. Terplan or any of the other members present to correct an impression of mine which is simply a clinical impression at this point. In the serial roentgenograms of children with early changes in their chest films, treatment with isoniazid seems to cause the lesions to disappear without the production of the large amount of calcium that we used to see in the cases that were simply treated by bed rest and observation, both in terms of the lesser appearance of the "Ghon tubercle" itself and in a smaller amount of calcified hilar lymph nodes.

I would like to know if that impression is correct or is simply an erroneous impression of mine.

DR. SCHMIDT (Cincinnati, Ohio): I would like to ask Dr. Canetti whether he has found qualitative differences in the pathology of the lung lesions of persons treated with isoniazid versus those treated with streptomycin.

I raise this question because of the work which we have been doing in induced simian tuberculosis which shows that there is a dramatic difference in both the lungs and the lymph nodes of animals which are treated with maximally effective doses of either drug.

In the persons who are treated with isoniazid, the evidences of tuberculous infection are almost uniformly absent, in both the lungs and the lymph nodes, whereas there is no difficulty whatsoever in determining that the individual had had previous tuberculous infection if he were treated with streptomycin.

DR. GIRDANY (Pittsburgh, Pennsylvania): I would like to address a question to Dr. Terplan.

First, I would like to ask Dr. Terplan whether in that single slide he showed of a large cavity there were, indeed, tubercle bacilli lining the cavity, or whether this was not an emphysematous bleb resulting from compression of the node.

Second (and this may be naive), is it fair to presume that, because we find viable organisms in the adrenals or elsewhere, this is the source of the hematogenous dissemination in miliary or tuberculous meningitis? Is it not also proper to examine, at least, the concept that there has been dissemination of tubercle bacilli during the initial infection through the blood stream to the central nervous system, and that something happens later to activate the bacilli already *in situ*.

DR. MCDERMOTT (New York, New York): I would like to address my remarks, Mr. Chairman, to the semantics involved in the question of chemoprophylaxis.

Dr. Canetti has distinguished between two types of situation, and I believe this is very important. It is important to realize that the use of chemotherapy in a situation in which the host has not yet taken tubercle bacilli aboard is, of course, true prophylaxis. It is that situation which has been studied by Dr. Palmer and Mrs. Ferebee in their initial studies with guinea pigs.

The more common situation in clinical practice, however, is the patient who has already taken tubercle bacilli within himself. In that situation the use of chemotherapy is really *treatment*; but the effects of the chemotherapy in crude, practical terms, may be really those of a "vaccination" or "prophylaxis."

Indeed, it certainly used to be true in the malaria field in the days of Atabrine®—and I assume it is true in most other fields—that there really is seldom such a thing as true chemoprophylaxis. What one is usually doing whenever one uses a drug in a chemoprophylactic situation is either suppressing the disease, on the one hand, until you get the soldier home; or actually treating it, on the other hand.

The fact that there are considerable theoretic reasons for believing that chemotherapy will not work as a truly prophylactic agent in a patient who has never had or never acquired tubercle bacilli is not to say that the use of chemotherapy in recently acquired tuberculous infection may not in substance accomplish the same thing as vaccination with attenuated tubercle bacilli.

DR. KASSOWITZ (Milwaukee, Wisconsin): I would like to ask this question: From the

viewpoint of "prophylactic chemotherapy" we have a primary lesion; don't we have to draw the line somewhere, let's say, according to age, arbitrarily at a certain age?

I am asking whether we are all convinced that up to a certain minimal age, let's say two or three years or pre-school age, we have to consider it a duty to go ahead and consider every minimal primary lesion as a symbol or a potential symbol? On the other hand, shouldn't we sit back, with a patient at the age of eight or ten years who is a positive reactor with minimal X-ray changes, and not use prophylactic chemotherapy?

DR. ESMOND R. LONG (Pedlar Mills, Virginia): I would like to say a few words about Dr. Terplan's paper, partly because I am very much indebted to Dr. Terplan. Quite a number of years ago, like Dr. Terplan, I was a student of Professor Ghon, and Dr. Terplan very kindly undertook to take me over the rough spots in an environment that was pretty unfamiliar to me.

I think we should recognize that Dr. Terplan is both the heir and the successor to Ghon, and it is no mean pair of shoes to fill. One of the most remarkable things about it is that Terplan is not only carrying on Ghon's work, but he has had the very great courage even to depart from some of the concepts of the old master himself.

One of them, in particular, is the one I want to discuss, namely the relation of the primary complex to subsequent infection. Dr. Dubos asked that we not discuss his paper, but I must refer to the fact that he did mention two or three of the very controversial points that we shall be taking up in this Symposium, one of them being whether primary infection is a protection in later life or not; and the other, whether primary infection is a possible source for tuberculosis in adult life.

That is a really bitterly debated point, perhaps more bitterly debated in the German school, from which Dr. Terplan came, than it has been in this country. It has never been settled, and I suspect it will never be settled, because I think tuberculosis itself is changing. If there is time, perhaps Dr. Terplan can come back to that.

I am impressed by this whenever I see pictures such as Dr. Terplan showed us, that childhood tuberculosis, as it is seen, is becoming—we might say—"at the top of the filter," a less serious disease. By the time it filters down to the post-mortem room, you will see a good deal of the old-fashioned tuberculosis with extensive caseation and ultimately with extensive calcification, such as Dr. Terplan showed us.

In between—and I am familiar with the material that Dr. Lincoln sees—the clinician still sees a great deal, of course. But this is not what you see at the top of the filter, where the filter material is most dilute. When a large group of people are more or less brought in, or are persuaded to come, or voluntarily come into a clinic, I don't think childhood tuberculosis is the sort of thing it used to be.

In the first place, as seen in the clinic, like the Phipps Institute and clinics of that character, there is far less active disease. That isn't surprising, because, of course, the sources of infection are considerably fewer than they used to be.

On the other hand, Dr. Terplan, I get the impression that, as you see it (and we see it mostly through X-ray films), it does not have the massive character of the tuberculosis that Ghon reported, that you yourself reported, and that we used to see so often.

In that very large Phipps Institute material, in recent years it has been quite unusual to see these tremendous lesions, and by the same token I think that spontaneous healing of the primary complex is usually much more thorough than it used to be.

So, I can well believe that, unlike the old lesions of a primary complex, which might have served not infrequently as the sources for tuberculosis in later life, because the post-primary dissemination was considerably more extensive, these much smaller lesions which Dr. Terplan is seeing now more than he used to might well heal so completely that it would be very unlikely that they would be the sources of subsequent endogenous infection.

I think Dr. Terplan's own work (and he is the man who has best shown it) demonstrates that this might actually be the case. He has shown repeatedly that one can get brand new primary complexes quite without any healing due to chemotherapy, just following spontaneous healing of the primary complex.

Dr. Terplan (Buffalo, New York): First, I wish to thank Dr. Long for his discussion. I am quite certain—and that's the reason why I tried to say what I presented today, which is perhaps only of historic interest—that we shall not see these severe changes any longer. I hope so. However, I showed a few pictures under the pressure of my friend, Dr. Middlebrook. One of the last pictures I had planned to show was the last case of massive tuberculosis which we experienced, a year ago.

That was just as classic as any one of the most really fulminating cases, fifty years ago. On the other hand, I also tried to state that such cases, and even the minimal infection, are, in numbers at least, definitely diminishing. I am fairly certain that they are of no great significance any longer.

Unfortunately, I have no experience to speak of the much more common type of tuberculosis in older children, and I am sure Dr. Lincoln has seen that. I have seen very many such instances in South America.

They all seem to have, at least according to the information I have received from pediatricians there, a better prognosis than the still classic focal lesions as far as tuberculous meningitis or massive miliary is concerned.

I am sure there are many exceptions to that general rule, and I would like to remind you again of a very old experience. I think Dr. Girdany was particularly interested in that—in correlation between the more extensive, chronic spread of the tuberculous process within the lung and the rather rare associated occurrence of significant hematogenous spread.

Of course, whenever resistance is lost, such a process can revert to a most severe progression.

I have had experience with a few surgical specimens, and it might interest you because I think the answer is, in part at least, in Dr. Long's discussion.

These resections were not performed for reasons of fear that the tuberculous process would spread. They were performed because, following these tuberculous lesions, older tuberculous lesions (both in bronchi and surrounding the bronchi) and bronchiectatic cavities have developed. It was very interesting to analyze such specimens.

They were perhaps in part of nonspecific origin. We could not demonstrate tubercle bacilli in the exudate from these cavities. They were all cultured. However, we could still demonstrate typical tuberculous processes in the firmly calcified lesions.

I brought a few extras slides, but they really don't show very much except the multiplicity of foci, with extension from the original lesions and with very calcified scars still in the draining lymph node.

Now, it is my belief—I cannot prove it—that the type of tuberculosis which we see now, particularly in the older children, which has the tendency to spread locally like the so-called infiltrative process, so well known in later life, in the adult, has less tendency to sudden or even selective hematogenous seeding than the classic, primary complex with the massive involved lymph node. This is the case even if some lymph nodes might be involved—as they always are if you have a chance to examine them.

Now, in answer to Dr. Girdany, the cavity which I showed in this case was a massive tuberculous cavity. It was diagnosed clinically as a diaphragmatic hernia. It was not diagnosed as tuberculosis. It was loaded with tubercle bacilli. Again, I was impressed by the massive tissue destruction. This is only one of a few cases of the former series which have no tuberculous meningitis and in which hematogenous seeding was most limited.

Also, the interesting lymph node involvement you might have seen in one of the photographs was comparatively small. So, even here, this strange "interlesion" may exist, but we have encountered many combined forms where it doesn't.

One, perhaps, is under the impression that in some of these cases the massive lesions actually prevented bronchogenic spread, from which the child died later.

I might not have made myself very clear in presenting the second case. Here, we were dealing with an old complex. I failed to demonstrate tubercle bacilli in an otherwise typical,

classical complex. However, I could demonstrate the primary lesion in the adrenal which was hematogenous, and from there the hematogenous extension was fairly clear.

What I can't understand is why in these cases so many organs are spared from hematogenous spread. That is really the problem which is in the last group I presented. It consisted of seven cases.

It is obvious that for many reasons I always believe tuberculous meningitis is the problem and that all of the other aspects of hematogenous tuberculosis in children are not so important. At least, that is a reflection of my experience over these last twenty-five years in Buffalo.

Dr. Davis asked a question about early roentgenographic changes which might disappear. I have no experience as far as that is concerned in cases treated with isoniazid. I have seen, of course, all the effects of such treatment in resected specimens from adults, but not from children. And I agree fully with most of what has been said by Dr. Canetti about these changes, particularly about the changes resulting from isoniazid treatment.

As far as tuberculous meningitis is concerned, Dr. Cocchi, I am certain that the advent of diffuse tuberculous meningitis is preceded by various other phenomena occurring more or less in a localized manner.

The question, of course, is what is first in this so-called hematogenous spreading of the process leading to tuberculous meningitis; and here we have entirely different figures from many others so frequently quoted in the literature.

That doesn't necessarily mean that one or the other observation is wrong. It so happens we couldn't find it. What I wanted to stress very strongly is that the alarming effects of tuberculous meningitis are not so much those of the setting up of an ordinary tuberculous process, but of allergy, if we can use that word, only comparatively speaking with other known allergic phenomena—it is the allergic apparatus which poses the problem here. This should be prevented, and it can be prevented only if you can prevent this massive diffusion of the tuberculous process with all that exudate infiltration. Or, let's say, one should aim at preventing those factors which give rise to the massive infiltration of arterial walls by all processes which occur in acute tuberculous meningitis because it is this vascular lesion, even when the treatment is antimicrobially successful, which always produces infarcts. Severe changes occur, not by the process of caseous tuberculous tissue formation, but by the process of arterial necrosis with infarcts.

Dr. Canetti (Paris, France): I will just say a few words.

I think I had better start with the matter of prophylactic chemotherapy. It is a very delicate one, but I think it is not the moment to go too deeply into it, so I think I won't answer any questions on that problem. I have only to answer Dr. Schmidt, I think, who asked me if I ever observed a major difference between cases—resection cases—treated with streptomycin and cases treated with isoniazid.

You see, Dr. Schmidt, this is a very difficult question to answer because the great majority of the patients whom I get, whom we see in France, have been treated with both drugs. I have few cases in which I note that the patients have only received either isoniazid or streptomycin, alone.

Our patients come to resection after long-term treatments which are not carried out according to a uniform scheme. So I have very few cases in which I can definitely say there has been no isoniazid or no streptomycin, and the question asked cannot be settled by the examination of a few cases because there are so many natural differences in reactions that you just have to have a lot of cases in order to come to any sort of statistically valid conclusions.

My impression is that the changes that I have described are a little more prevalent with isoniazid in combination with other drugs than with isoniazid alone, but I wouldn't give this as an absolutely sure statement. I haven't examined enough cases. I think this should be left open for future research.

There is another difference between your observations on monkeys and those which I

have made on human beings. That is, in the first place, that you could give them but one drug or, at least, you knew when they had no other drug and which drug they had. But the major point is that you have given them a far higher dosage than human beings presently get, so it is possible you have seen far more clear-cut differences due to that dosage than we could see with the dosages we have used.

I must add that one of the first cases described with those extensive numbers of giant cells was a case described by Dr. McDermott and his associates as early as 1947 or 1948, and without any isoniazid treatment.

DR. DAVIS: I want to ask Dr. Terplan a question. He showed in his paper that death is very commonly due to the results of hematogenous tuberculosis. Now, this is an observation acceptable to anybody studying the disease in children. We have made a survey of 100 consecutive deaths in children in London and found that in 92 per cent of them, the death was due to hematogenous dissemination.

Studying children with primary infection, we have found that these hematogenous forms of tuberculosis differ with age, that it is much higher in infants and then progressively gets lower. In the textbooks we read that hematogenous dissemination occurs in every case of tuberculous infection. This statement in the textbooks seems to be based mainly on bacteriologic observation when bacteriologists, early in this century, cultured tubercle bacilli from urine or blood of subjects with tuberculous infection. But we must not forget that the same observers often cultured them from cases of rheumatoid arthritis or other unrelated conditions.

My specific question is: Does hematogenous dissemination invariably occur in every case of primary tuberculous infection?

DR. TERPLAN: I can answer that very briefly. It does not. There were many incidental findings which were of strictly morphologic interest to pathologists but which were very helpful in studying the question of pathogenesis, and in which the tuberculous nature of these lesions could be proved to be restricted to an area of localized infiltration with or without lymph node component.

I do believe that there is a certain relationship between age and progression of tuberculosis by the hematogenous route. It is very difficult to discuss that today because the whole picture has changed.

Ten or fifteen years ago I would have said that naturally occurring acute hematogenous tuberculosis in primary infection of an adult, even with the process locally spreading, was much more uncommon than, let's say, the usual intrabronchial spread with restriction to the lung. However, we have seen it, and I have included some of those observations in the acute progressive stage; but the number of those is limited in comparison to the large number of cases of chronic tuberculous disease without any appreciable hematogenous spread.

The picture of chronic tuberculosis of the lung in the adult is still a great problem of pathogenesis and is entirely different from what I have seen in infants and children—which is still most complicated. I am not so sure but what I might add a word to my answer to Dr. Davis.

May I ask Dr. Davis whether he was satisfied or not?

DR. DAVIS: I think possibly the answer might come out later in the discussion. Some of the people here have had more clinical experience on this point and may be able to answer it more fully.

DR. TERPLAN: I think the only reason I can't answer that question in the way you meant it is because most of the lesions which I saw as incidental findings were old lesions.

DR. EDWIN L. KENDIG (Richmond, Virginia): I would like to comment on the question that Dr. Long and Dr. Terplan both brought up, to the effect that tuberculous infection in childhood is considerably decreased. I think we will all agree with that as a general statement. It is certainly clear, however, that in certain localities it isn't true, and Richmond happens to be one of those places.

In a study which we have been conducting for the past six years, we have found in a control group that 14 per cent of children have been infected by eleven years of age and 1 to 2 per cent by the time they reach two years of age.

DR. DUBOS: I didn't understand what Dr. McDermott meant by introducing the word "chemoprophylaxis." There is no semantics problem, as I see it. Perhaps he could elaborate on what he meant.

DR. CANETTI: I have no explanation to add. I think Dr. McDermott just tried to give a statement of the different types of chemoprophylaxis: one being used—that is, being initiated—in the uninfected patient and the other directed toward already existing latent infection in an attempt to prevent the transformation of this infection into real disease.

Is this so, Dr. McDermott, or did you mean something else?

DR. MCDERMOTT: I believe that your explanation of my position is the correct one, Dr. Canetti.

I simply did not wish to leave the implication that the same theoretic objections to the use of isoniazid before tubercle bacilli had been acquired by the host, or even at a certain time thereafter, necessarily applied to the use of isoniazid in children who had become tuberculin reactors. In substance and in terms of practical results, the latter procedure is really the same thing as prophylaxis but, strictly speaking, it is treatment.

DR. CANETTI: I agree that they are different problems. I, at least, am always thinking of the problem of chemoprophylaxis in terms of conditions which really don't exist in any Western countries but which do exist in Eastern countries—in India and Pakistan and China, and so on. A terrific amount of tuberculous disease exists there, and the absence of any possibility of getting most of the patients into the hospital and of breaking contact between the child and the diseased adult carrier are real problems. This is a dramatic situation which faces the public health people who have to deal with it.

Vaccination is good, but we know vaccination can't give protection before three months or, perhaps, even later; during these three months, a lot of things may happen.

Of course, treating the patients may cut down the volume of sputum and diminish the number of bacilli; but still, the infection risks are very heavy. So there is, of course, a great temptation to give isoniazid to these children, exposed to contact cases, and thus to prevent immediate contamination.

But the question remains whether isoniazid *alone* can afford any protection after it has been stopped. I must say I entirely share Dr. Dubos' views, although we have to obtain more evidence. I can't believe that isoniazid prophylaxis can leave behind any amount of immunity after administration of the drug is stopped. I don't see how this could possibly be, because isoniazid necessarily stops bacillary multiplication, and there is no antigen available to build up such an immunity in the host. Therefore, isoniazid, while probably a good measure, doesn't work for the future. One must consider the future. This is the reason why I advocate the administration of isoniazid-resistant BCG vaccine to all the exposed and still uninfected children: they would build up a specific immunity unhampered by the simultaneous administration of isoniazid; and thus be protected—as far as vaccination projects for the future are concerned.

## Session 2

Wednesday Afternoon, November 9, 1955

EDGAR MAYER, *Honorary Chairman*

JAMES J. WARING, *Moderator*

# VACCINATION AGAINST TUBERCULOSIS

### Clinical Aspects

LARS STRÖM[1]

In combating tuberculosis one has to lay the greatest stress on prophylaxis. We have to realize that BCG vaccination is only one of several measures that can be applied in order to protect children against tuberculous disease. We know that BCG vaccination cannot prevent tuberculous infection, but that it does diminish the risk of "virulent disease" after the first infection and may, therefore, be called, according to Wallgren, "specific disposition prophylaxis." By unspecific disposition prophylaxis, on the other hand, we mean a general elimination of everything unnatural, unhygienic, and unwholesome in the child's diet, care, and development. In judging the value of BCG vaccination, one must remember that about twenty-five years ago, when BCG was introduced in several countries, the situation with regard to tuberculous disease was quite different from what it is today. No doubt BCG vaccination was once a welcome and important means in the struggle against tuberculosis, especially for the pediatricians. At that time the mortality among children infected with tuberculosis during their first year of life was 35 per cent (in Sweden). Now we know that such children, vaccinated generally, are free of clinical tuberculosis and we never see a case with post-primary clinical manifestations of tuberculosis.

Many studies on the effectiveness of BCG vaccination have been published. I would briefly cite some of the satisfactorily controlled investigations. In the United States, Aronson carried out a study of BCG vaccination among the Indians on the reservations in the West, where tuberculosis was a serious infection. In the group of 1,857 tuberculin-negative persons, 185 developed tuberculosis, with 28 dead during the time of observation. In the group of 1,551 vaccinated persons, 5 developed clinical tuberculosis and 4 of them died. In his study of nursing students, Heimbeck in Norway found a morbidity of 29.6 per cent among those who were tuberculin negative. Among those who were tuberculin positive, either because of previous tuberculous infection or after a BCG vaccination, tuberculosis developed in only 2.6 per cent and 2.3 per cent, respectively. In Sweden, Dahlstrom and Difs carried out an investigation of conscripts inducted into military service. One hundred and thirteen thousand, four hundred and ninety-two persons were tuberculin positive and 61,774 were negative. Of the negatives, 36,235 were vaccinated and 25,239 served as the nonvaccinated controls. In the group of vaccinated persons, 14 developed primary *uncomplicated*

[1] From Karolinska Sjukhuset, Sweden.

tuberculosis, while 27 in the nonvaccinated group developed tuberculosis. In the vaccinated group, 76 persons developed primary tuberculosis complicated by pleural effusion, compared with 115 persons in the nonvaccinated group. Equivalent degrees of progressive pulmonary tuberculosis were developed in 8 of the vaccinated group and in 12 of the nonvaccinated group.

The results of these investigations are perfect arguments in favor of the effectiveness of BCG vaccinations, i.e., a BCG vaccination produces a specific immunity that increases the resistance to any virulent tuberculous infection. In other words, when people are vaccinated with BCG and are then exposed to tuberculosis under similar circumstances they are, no doubt, better protected than nonvaccinated persons.

In order to achieve an effective vaccination, the vaccine must be given by a proper technique. Three methods may be taken into consideration. The oral method used in the first experiments with BCG was, for a time, abandoned; but at present, and for several years now, it has been the method of choice in many South American countries. The intradermal method is the one which has been used most generally. The third method is the percutaneous or the multiple-puncture method.

With the intradermal vaccination we are using 0.05 to 1 mg. of dried vaccine in a solution of 0.1 ml. injected intracutaneously after preliminary sterilization of the skin. The vaccine can be introduced in the deltoid area of the arm in adults or, as we regularly do it in Sweden, into the skin over the lateral aspect of the left buttock. This intracutaneous injection leads to the development of a small localized infiltration, which sometimes may produce a superficial necrosis of the skin; sometimes, a well-marked ulcer. In association with this, there may be an enlargement of the regional lymph nodes, which very rarely develops into a suppurative inflammation. In the percutaneous methods of Birkhaug and Rosenthal, the vaccine (containing 20 mg. of BCG per ml.) is applied to the skin, using the Birkhaug or the Rosenthal instruments. The vaccine is introduced into the skin by means of a number of small sharp points or small knives, respectively. These methods are quite painless and leave practically no scars. By either the intradermal method or one of the percutaneous methods, equivalent success can be obtained.

The local reaction after the intradermal method seems to depend upon different factors. Of these factors, the dose of vaccine is of less importance than the technique of injection. If a subcutaneous injection is made, the risk of necrosis or even subcutaneous abscess is much greater. This may depend upon what happens with the vaccine after injection. Probably a drainage of the vaccine takes place, at least partly through the lymph vessels as far as the regional lymph nodes.

By the aid of a BCG vaccine labeled with radioactive phosphorus ($P^{32}$), I have studied on the skin surface the dispersion of this vaccine and this dispersion seems to take place along the lymphatic channels (1).

After ten to fifteen minutes, in small children the activity is traceable for a distance of about 10 cm. over the inguinal glands, provided the injection is made in the thigh. This is the case in tuberculin-negative subjects and persons not immunized. When, on the other hand, the injection is performed on tuberculin-

positive, BCG-vaccinated subjects or on persons who have had tuberculosis previously, it will take much longer before any radioactivity outside the papule itself can be measured, if at all.

In experiments on guinea pigs, using exactly the same technique, identical differences are obtained in the curves.

I have also studied the urinary excretion of $P^{32}$ in vaccinated and nonvaccinated animals injected with labeled BCG. The dissemination of the bacilli takes place very rapidly in the normal animal. Within an hour we can measure the activity in practically all tissues and organs. Another observation we have made is that the elimination of $P^{32}$ is found to be significantly increased in the immunized animals. This might be interpreted as an indication of immunity. This urinary excretion has also been confirmed recently by Sternberg in Montreal (2, 3) and also at the Centre International de l'Enfance in Paris, where studies with radioactive vaccine have been carried out by Pasquier (4).

About the vaccine used: I suppose all current attempts at artificial immunization against tuberculosis utilize either the BCG vaccine or the vole vaccine. But killed bacterial vaccine and extracts of tubercle bacilli have also been utilized in trying to produce artificial immunity. Such a killed bacterial vaccine is used in Italy, according to the Salvioli-Petragnani method. With this vaccine the local skin reaction is much bigger, causing ulceration and necrosis. I have tried to compare the immunity after using normal BCG vaccine and a killed bacterial vaccine according to the above mentioned isotope technique. I can only say that the arrest of the radioactivity in the inoculation site of infected persons or previously vaccinated subjects seems to be less with the killed bacilli than with the living BCG. On the other hand, the resistance against tuberculosis of a group of guinea pigs vaccinated by the method of Salvioli-Petragnani seems to be the same as in a group of animals vaccinated with BCG.

The oral method, as I said, is the method of choice in South America. I have not found, in the literature available, experimental data as a basis for the effectiveness of this method. During the past year we have tried, using the labeled vaccine, to get an impression of the effectiveness of immunization after oral vaccination of guinea pigs. In Montreal, I have now started experiments with Dr. Sternberg to penetrate this problem. To this date, I think I am able to say:

(*1*) That the immunity after the oral vaccination is more uncertain.

(*2*) That, in those cases in which we find an active immunization, it is due to absorption through the mucous membranes of the mouth and not through the intestines.

* * *

Now, what may BCG vaccination accomplish at best? This very important question of the effectiveness of BCG vaccination brings up the problem of the degree of artificial immunity produced by BCG. I think that, at present, nearly all physicians who have considered the matter at all agree that BCG vaccination really is able to produce a specific immunity, increasing resistance to virulent tuberculous infection. I think I have already mentioned that arguments in favor of this view have been presented by the above cited authors, among others. What is, then, the importance of BCG in explaining the present low morbidity

and mortality? As I have already pointed out, the BCG vaccination is only one of several factors applied to protect children against tuberculous disease. Which are, then, the other factors? We think that in Sweden the following reasons for reduction in morbidity and mortality may be noted:

(*1*) Increase of resistance to tuberculosis due to general biologic factors and to general hygienic conditions.

(*2*) The most delicate ages of childhood are less exposed by a decrease in sources of infection. This is due to:

(*a*) Rational organization of dispensaries

(*b*) Improved public enlightenment

(*c*) Increased availability of hospital beds

(*3*) More effective treatment, including chemotherapy and surgical treatment.

(*4*) BCG vaccination.

Recently, Wallgren has discussed the virtues of mass vaccination with BCG and, concerning the future of the BCG vaccination, I will refer you to his paper.

It is evident that among all of these aforementioned factors BCG vaccination in the form of mass vaccination has lost more and more of its importance. The value of BCG vaccination varies, of course, under different circumstances. If we look upon the problem of today, the indications for mass vaccinations are quite different: on the one hand, in countries where primary infections develop early in childhood and where the manifest forms of primary tuberculosis and the early post-primary disease are common; on the other hand, in countries where the primary infection takes place in childhood in a very small number of cases, which is the situation in the Scandinavian countries today. In countries with late primary infections and satisfactory nonspecific resistance, the great majority of BCG vaccinations will never exert a protective influence during childhood; they become relatively unnecessary.

Finally, there is no question about the need, use, or value of individual BCG vaccination in cases in which the danger of exposure is particularly marked, e.g., in children living in tuberculous surroundings or among people engaged in certain trades. It is neglectful not to vaccinate tuberculin-negative children when any relative suffers from pulmonary tuberculosis, even should it be regarded as no longer active. It should be considered equally erroneous to omit the vaccination of student nurses or medical students, or the staff of a sanatorium or other hospital or institution to which people with tuberculous disease may be admitted.

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# USUAL AND UNUSUAL REACTIONS TO BCG INOCULATION IN CHILDREN

HANS JACOB USTVEDT[1]

The usual reaction of the child to inoculation with the attenuated strain of *Mycobacterium tuberculosis* called BCG consists in a grossly visible primary focus in the skin and a less easily ascertainable regional adenitis.

In nonallergic subjects the local tissue response shows considerable variation in course, extent, and intensity, and numerous factors in the host-parasite relationship influence the quantitative and the qualitative aspects of the local response.

Palmer and associates (1) have demonstrated the importance of the total mass of bacterial cells injected, of the number of living cells injected, and of the relation between living and dead organisms in the vaccine. Large-scale investigations in animals, as well as in children, point toward the dubious significance of the local vaccinal lesion as an index of a vaccine's immunizing potency. Dubos and Pierce (2) maintain that the extent and duration of the local lesions produced in the skin of guinea pigs provide convenient criteria of differentiation between various substrains of BCG.

With regard to the host, age is of some, and sex of very little, importance. Nissen Meyer and Palmer (3) have found slightly lower tuberculosis death rates in families whose children showed a rapid development of vaccinal lesions, compared with those who developed local lesions more slowly. These differences, although statistically not significant, are in accordance with animal experiments by Aronson and Sokoloff (4) and Lurie and associates (5), indicating that the rate of development of the vaccinal lesions may serve as an index of hereditary resistance to tuberculosis.

## *Local Tissue Response*

The local tissue response is more severe (with a stronger tendency to abscess formation) when the bacilli are inoculated subcutaneously than when given intracutaneously.

Regarding the influence of nontuberculous disease, it has been maintained that an unusual type of local response is encountered in sarcoidosis. Israel and associates (6) and Bjornstad (7) have found that the local lesion in the majority of sarcoid patients is indistinguishable from that observed in controls.

In allergic subjects, inoculation of BCG will usually produce an accelerated inflammatory response which may rapidly develop into productive tubercles or necrosis. The transition between the classic Koch phenomenon with ulceration, elimination of the bacilli and rapid healing, and the accelerated nodule formation without sloughing is gradual. Accelerated local response is (frequently) observed in mass vaccination campaigns, because no tuberculin test can separate the subjects into two mutually exclusive groups—those with and those without a

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definite sensitivity to tuberculin. In addition, this type of response is seen in revaccinations.

The accelerated local response may be accompanied by a flare-up of the site of a previous tuberculin test. In more pronounced cases, fever and general symptoms may develop. Whether an activating influence may be exerted on the pre-existing tuberculous infection is an open question.

Of particular interest is the fact that an accelerated local response to BCG with necrosis may be accompanied by a definite, and sometimes suppurative, regional lymphadenitis in contradiction to current views regarding lymph node response in superinfection (8).

### *Lupus Formation*

An unusual type of response to BCG inoculation is *lupus formation* at the site of vaccination. Some thirty cases have been described, fifteen from Scandinavia. The clinical findings seem typical of lupus vulgaris, with lesions up to 100 sq. cm. in area. In most cases an extensive local lesion has persisted and increased during months (sometimes years), with gradual development of a lupoid aspect, with an interval of a few months up to several years from vaccination to diagnosis. Most cases have presented definite lymphadenitis, some with suppuration. All patients have responded well to treatment.

The age distribution is quite remarkable, eleven of eighteen patients between thirteen and seventeen years of age—at or near puberty.

Sensitivity to tuberculin was mostly strong or moderate. Very little (if any) evidence of exposure to virulent human or bovine infection was reported. The histologic picture has not differed from what is usually found in ordinary lupus. In four cases, mycobacteria showing eugonic growth on Löwenstein medium and being nonpathogenic for guinea pigs have been cultivated from the lupus lesion; and in another three cases, from the accompanying lymphadenitis.

There is little reason to doubt that BCG has been the agent, at least in the majority of cases. Development of lupus in a cutaneous primary focus is well known from pathology studies of tuberculosis in humans, and ordinary lupus often develops from scrofulodermata. Lupus nodules at the site of the local lesion have been reported to occur not infrequently after vaccination with the vole bacillus, and have also been observed in lesions produced by the "swimming-pool bacillus" (*Mycobacterium balnei*).

The finding of attenuated mycobacteria does not in itself constitute proof that the lesions are caused by BCG. It is well known that strains of tubercle bacilli cultivated from ordinary lupus lesions are frequently attenuated. According to K. A. Jensen (9), it is, however, relatively easy to distinguish between lupus strains and BCG. The former give more pronounced lesions than BCG on intracutaneous inoculation in guinea pigs and show ample growth on Besredka's medium, in contrast to BCG. In at least one of the cases of lupus at the site of BCG inoculation, Jensen has succeeded by the above means in identifying BCG.

We know next to nothing of the reasons why these particular subjects have reacted to BCG inoculation with the formation of lupus nodules. Relevant factors

may probably be operating mainly on the side of the host and the explanation may be highly complex. In one case the patient's mother had suffered from ordinary lupus. In another case, the local lesion had healed but had relapsed in connection with measles and developed into lupus. Of particular interest is the observation that in six of fifteen cases the lupus occurred at the site of re-vaccination, and, in two of these, the re-vaccination had been performed at exactly the same spot as the first vaccination. Further, in four, and possibly in six cases, the lupus developed at the site of a typical Koch phenomenon.

Lupus formation in vole vaccination has been ascribed partly to trauma and to secondary infection. Very little evidence is presented in the BCG cases regarding the influence of such factors.

We probably will have to take into account a multiple causation: genetic factors, temporarily diminished resistance due to intercurrent infection, possibly secondary infection of the skin, and particular circumstances regarding hypersensitivity.

Lupus was the only unusual response to BCG detected in all of these cases except one, in which multiple, benign foci in the skeleton were disclosed and the finding in one of the foci of attenuated mycobacteria, nonpathogenic for guinea pigs.

### *Lymph Node Involvement*

The quantitative and the qualitative variations in the lymph node response to BCG inoculation are less well known because they are not easily ascertained. Important studies, however, have been published by the Pilot Station of the International Children's Center in Paris and by the Tuberculosis Research Office of the WHO in Copenhagen. There is every reason to assume that we must accept a range of individual variations like those observed for the local tissue response. Caseation with abscess formation may represent only the most intensive response within the range of "normal" reactions, but the fact that pre-existing inflammatory conditions in the lymph nodes may influence the course in some cases cannot be entirely excluded.

With regard to the vaccinating agent, it has been established that the dose of vaccine employed, and probably the number of living germs, are of paramount importance. The claims made that different substrains of BCG differ with regard to their tendency to produce suppurative lymphadenitis have so far not been substantiated by systematic studies in which the dosage and other variables have also been taken into account.

Among the host factors, age is of decisive importance, with a much more pronounced tendency to extensive lymph node reactions in infancy and early childhood, similar to that seen in natural tuberculous infection.

Histologically, the lymph nodes show typical tuberculous tissue response, often with caseation. Calcification has been observed roentgenographically. The lymph node reaction may affect not only the strictly regional lymph nodes, but groups in the vicinity, and, rarely, ectopic lymph nodes at some distance (parasternal lymph nodes in vaccination on the forearm; intra-abdominal nodes in vaccination on the thigh).

Bacteriologically, mycobacteria which behave in culture and animal inoculation like typical BCG have repeatedly been disclosed in the lymph nodes involved after BCG vaccination. The presence of pyogenic bacteria seems to be rare. Sensitivity to tuberculin is strong principally in cases with suppurative lymphadenitis. A slightly positive correlation seems to exist between large local lesions and pronounced lymph node reactions, but suppurative lymphadenitis may be seen in cases with small local lesions, and vice versa.

It is possible that the mediastinal hilar lymph nodes may be involved through spreading via the lymph channels, but definite proof of this is lacking. The claim that BCG may cause bilateral hilar adenitis and sarcoidosis is not sufficiently well founded.

### *Hematogenous Dissemination of BCG*

There is some indirect and some direct evidence that a *hematogenous dissemination* of BCG, with formation of minute, regressive foci in several organs, is a usual occurrence in connection with cutaneous BCG inoculation in human beings.

In analogy with what happens in "virulent" infection, we may also expect such a dissemination to take place after inoculation with attenuated strains. Studies in animals with BCG labeled with radioactive phosphorus point to a rapid spreading of the radioactive substance throughout the body. BCG has been cultivated from the lung tissue of normal guinea pigs after intradermal inoculation, and minute regressive nodules have been observed in exceptional instances in animals used for the control of BCG vaccines.

Direct evidence of hematogenous dissemination of BCG in human beings has recently been presented by Gormsen (10). He has carefully studied the organs of twenty-six children who were vaccinated with BCG and who died suddenly, the investigation being made in conjunction with medico-legal autopsies in Copenhagen.

In the majority of cases, definite epithelioid cell granulomata were found in several organs, particularly in the liver and the lungs, with giant cells of the Langhans' type, without necrosis, and with no detectable tubercle bacilli. In two infants a considerable number of these granulomata was found. Control examinations of children who have not been inoculated with BCG have failed to disclose similar foci. The bacteriologic proof that these granulomata were due to a hematogenous dissemination of BCG is still lacking, but the evidence in favor of such an assumption must be regarded as strong.

With regard to disease detectable *in vivo* and due to a hematogenous dissemination of BCG in human beings, a small number of cases has been reported in which benign foci have been disclosed in the skeleton or the subcutaneous tissue. The bacilli cultivated from such lesions have shown typical eugonic growth and no pathogenicity for guinea pigs (11–14). The most impressive is the lupus case mentioned earlier, with roentgenographic signs of foci in five different bones.[2]

Of quite different importance, however, are four fatal cases of generalized

[2] Case 3.

disease resembling tuberculosis in BCG-vaccinated individuals, in which the authors claim to have identified the agent as BCG (15).

*Fatal Infection with BCG*

Only a brief summary will be given of the salient points concerning these fatal cases. Two cases have been reported from Sweden (16, 17); one from Denmark (15), and one from Norway (18). Two of the patients were infants, the other two being seven and nineteen years of age; three were female, one male. There was no tuberculosis known in the family, and no known exposure to virulent infection, human or bovine. Three had been vaccinated intradermally and one by multiple puncture. They all developed detectable lymphadenitis, three with suppuration. Sensitivity to tuberculin, tested during the illness, was weak or lacking.

The clinical picture was not uniform. In a girl of nine months (16), vaccinated at birth, suppurative lymphadenitis was immediately followed by fever, poor general condition, and generalized swelling of the peripheral lymph nodes, but no lesions were clinically detectable in the lungs or the skeleton. The child died at the age of fifteen months after six months' illness.

In the second case (15), a boy of seven, the disease started with generalized swelling of the lymph nodes three to four months after vaccination. It lasted for two years, presenting the typical, nearly forgotten picture of a severe, malignant form of scrofulosis, with multiple lymph node abscesses all over the body, some with fistulization, and with episodes of high fever.

In the third case (18), of a man aged nineteen, the first lesion detected was a regional suppurative lymphadenitis one year after vaccination, followed by an abscess of the chest wall. The disease lasted for four and a half years, with successive foci in the lymph nodes, the spine, the ribs and other parts of the skeleton, the lungs, the pleura, and the kidneys. A serosal type of meningitis developed after two years but regressed.

In the fourth case (17), a boy of six months, vaccinated at birth, was in a poor general condition, with respiratory difficulties and roentgenographic signs of large, progressive lesions in both lungs. The course in this case was a rapid one, with death on the twelfth day after hospital admission.

In all cases, treatment seemed to be of only temporary, if any, avail. All of the patients received streptomycin, and two of them also received isoniazid.

Biopsy was performed in three cases, and autopsy in all four, and the pathologic findings have been described in detail for three cases. *Macroscopic* evidence of lesions in lymph nodes and lungs were found in all cases; foci in the skeleton, in only one case. A few discrete lesions were seen in the spleen, the kidneys, the liver, and the thymus. In the first case, the autopsy disclosed, beside the lymph node enlargement, disseminated abscesses in most of the organs. The diagnosis was miliary tuberculosis. The central nervous system is mentioned in only one of the reports as normal.

*Microscopically*, one finding was common to all four cases: the almost complete lack of definite tubercle formation. A more or less diffuse infiltration was found,

with varying amounts and arrangements of epithelioid cells, leukocytes of different types, macrophages, plasma cells, and giant cells (both of the Langhans' and of the foreign-body type). Caseous necrosis with abscess formation in the lymph nodes was found in two cases; it was lacking in the other. Enormous numbers of acid-fast rods were seen in some foci in three of the cases: partly in the caseous material; partly in the granulation tissue.

In the boy of seven, the pathologist (Professer Engelbreth-Holm) drew attention to peculiar lesions in the lungs, with thickening of the alveolar walls, numerous cells, and diffuse, amorphous calcium deposits in the connective tissue stroma, with no tubercle formation and no tubercle bacilli.

In the third case, Waaler stresses the finding of numerous plasma cells in the spleen and lymph nodes, with marked pyroninophilia. In the spleen of this patient there were also large amounts of a yellow pigment which gave a positive iron reaction.

The common denominator in the three well-described cases seems to be the absence of tubercle formation, the diffuse granulation tissue with various types of cells, and the myriads of acid-fast rods visible in some foci and lacking in others. From the pathologic point of view, the findings might be grouped under the heading "atypical tuberculosis."

In the *bacteriologic* investigations, mycobacteria were obtained in the first case from one lesion and in the others from several lesions, partly *intra vitam* and partly *post mortem*. In all four cases the strains showed eugonic growth on Löwenstein-Jensen medium and did not produce generalized tuberculosis in guinea pigs. In two cases no isoniazid had been given; in the other two, several bacteriologic investigations, with the same findings, were performed before isoniazid treatment and during a period of time when treatment with isoniazid had not yet started in the Scandinavian countries.

Of particular importance is the observation made in three of the cases, that the strains did not grow on Besredka's medium. According to Jensen, both the attenuated, isoniazid-resistant strains of tubercle bacilli and the attenuated lupus strains showed ample growth on this medium.

Pathogenicity for rabbits was tested in two cases—with negative results. "Cord formation" was studied in two cases, and none of the strains showed the typical cord formation of ordinary virulent strains. This is particularly stressed by Oeding for the third case, in which sixteen samples were studied in slide culture. They formed unoriented colonies "completely different from the cords seen in human and bovine strains." In the fourth case a number of supplementary examinations were made. The strains did not grow at 22°C.; they grew in Dubos fluid medium and on Dubos oleic-acid–albumin–agar medium. Like BCG, they were catalase positive; they were as susceptible to isoniazid as is H37Rv. With neutral red the strains isolated became colored faintly red, and the aryl-sulfatase reaction was negative.

From the second case, thirteen strains have been studied elaborately by Jensen, all showing uniform conditions of growth and virulence similar to BCG.

In all four cases the bacteriologists concluded that the germs isolated from the

different foci, both regarding morphology and pathogenicity, were indistinguishable from BCG. This conclusion seems to me to be well founded. The agent operating in these cases has obviously been an attenuated strain of mycobacteria, with the same degree of attenuation as BCG, and there is nothing in the bacteriologic findings against the assumption that it may be BCG itself.

In other words, extensive lesions resembling atypical tuberculosis have been disclosed six to twelve months after vaccination in persons who have been vaccinated with BCG, with no anamnestic or pathologic evidence of virulent tuberculous infection. From these lesions, tubercle bacilli have been obtained which could not be distinguished from BCG.

In my opinion, one cannot escape the conclusion that the widespread and fatal atypical tuberculous disease in these subjects must have been related to BCG, which had disseminated hematogenously and possibly lymphogenously to create progressive lesions in several organs.

Is there any other acceptable explanation? The possibility of an infection with isoniazid-resistant strains coinciding with the BCG inoculation can (according to what I have said) be discarded. In recent years an increasing number of cases has been reported where atypical mycobacteria have been obtained from lesions of a tuberculous type in human beings, and some of them have been nonpathogenic for guinea pigs. Most of them are chromogenic, but cases are also known in which nonchromogenic bacilli have been cultivated. Such cases, however, seem to be rare.

It would be highly artificial, in my opinion, to postulate that the above cases represented a coincidence of BCG vaccination and infection by atypical mycobacteria of virtually the same degree of attenuation as BCG. It is not known that any of the patients had visited swimming pools, and for the infants this possible exposure can certainly be excluded. Moreover, the bacteriologic findings do not tally with *Mycobacterium balnei*. For similar reasons, infection with avian strains must be discarded.

If we accept BCG as the agent in the cases concerned, we must also admit that no evidence has been produced to the effect that BCG in these cases has shown any increase in its intrinsic virulence, either in the laboratory or in the host.

For three of the cases we are informed that groups of children were vaccinated at the same time, not only with the same batch of vaccine but also with vaccine from the same ampoule, without any complications having been reported. The control tests in the laboratory have given the ordinary, negative results. In two cases, the same strains have been isolated at different periods of time during the course of the disease; and no increase in the intrinsic virulence has been discovered in the animal experiments.

Thus, it seems as if ordinary BCG, with the usual degree of attenuation, has been inoculated into the subjects concerned. Why have these few among the millions of subjects vaccinated given this peculiar and tragic response? A perusal of the details given in the papers throws very little, if any, light on this problem.

With regard to possible *genetic* factors influencing nonspecific resistance, we note that in none of the cases had tuberculous disease occurred in the family. The fact that all four cases have been observed in Scandinavia could hardly be

interpreted as evidence in favor of a racial lack of resistance; but more naturally it could be taken as an indication of the relatively good conditions for close observation in those countries.

Wallgren (19) has suggested the possibility that these exceptional cases have occurred in subjects who suffer from a congenital deficiency in gamma globulin. However, in the one case in which electrophoresis of the serum was performed, a percentage of 44 for gamma globulin was found, a value which is more than twice the normal. In the lupus case mentioned, with lesions of the skeleton, normal values were found for gamma globulin.

Similarly, very little positive evidence in favor of a temporarily decreased, nonspecific resistance is available. No details are given with regard to diet. None of the patients had been exposed to silica. They had not been subjected to other immunizations in the period between vaccination and disease. There is no evidence pointing to a simultaneous viral infection. In the first infant, a poor general condition was observed when the child was admitted, but we do not know whether this was the result of the disease or not.

The boy of seven years of age had gone through the ordinary diseases of childhood earlier and was in good general condition. The man of nineteen had suffered from the acute infectious diseases of childhood, from acute appendicitis, sinusitis, and pneumonia, and was reported to have had, since childhood, an erythrocyte sedimentation rate of about 40 mm. on repeated examinations. We could not tell what this may have meant for his resistance. However, the electrophoretic examination showed that he did not lack the ability to produce gamma globulin.

In this case considerable amounts of iron-containing pigment were disclosed in the spleen, and Waaler mentions the possibility that he may have suffered from some sort of hemolytic disease. However, numerous blood examinations were made during this disease, and nothing peculiar was disclosed apart from an ordinary secondary anemia, with a hemoglobin content down to about 50 per cent of normal.

The second infant, six months old, had suffered from generalized eczema and rickets, and presented a poor general condition; and in this case one may assume that the nonspecific resistance was low.

Nothing in the laboratory findings seems to throw any light on the problem. Positive serologic tests for syphilis (Wassermann and Kahn reactions) in the first infant may be interpreted as false positive reactions, since the Meinicke reaction was negative and the mother's serum gave a negative Wassermann reaction.

Even though the information available does not permit us to single out certain factors influencing resistance, such factors may of course have escaped notice. A multiple causation may have been present, involving dietary factors, infections, and other undetected diseases beside possible genetic factors.

I will not venture upon speculations regarding a possible lack of capacity to develop immunity in these cases. Certain facts should be noticed. Cutaneous sensitivity to tuberculin was found to be weak or absent in all four cases. None of the patients showed any important response to treatment. In one of the cases

the bacilli became resistant to streptomycin, but in one case they remained susceptible in spite of the lack of response.

Waaler interprets the increased gamma globulin content of the serum in his case, and the marked increase in plasma cells, with remarkable pyroninophilia as indications of a "strong immunity reaction" and as a most astonishing finding in a person who seemed to lack the ability to wall off the inflammation, and who was eventually killed by the disease.

However, it is questionable whether one is justified in interpreting an increased gamma globulin content in the final stage of tuberculosis, which is a common finding, as an indication of immunity. Most of the increased gamma globulin is probably not antibody. Good (20) maintains that persons suffering from deficient production of gamma globulin do not develop plasmocytosis in response to even the most intense antigenic stimulus, whereas the hypergammaglobulinemia in the final stage of tuberculosis often is accompanied by an increase of plasma cells in the bone marrow.

The variation of the histologic findings in the four cases is difficult to interpret. To some degree they remind one of the findings in *sepsis tuberculosis acutissima*. The enormous number of acid-fast rods found in certain foci may recall both this condition and the picture seen in certain cases of disease presumably caused by atypical mycobacteria.

While it has been maintained that these four subjects were entirely lacking in resistance to tubercle bacilli, and that they would rapidly have fallen prey to a natural tuberculous infection, I doubt whether this conclusion is justified. What we know is that they lacked resistance, for some reason or other, to BCG. But we cannot tell how they would have reacted to an ordinary tuberculous infection which would probably have taken place with a much smaller infecting dose and might have called forth much stronger reactions of immunity.

A final point: Do these four cases represent the extreme within a continuous range of human reactions to the hematogenous dissemination of BCG, or is it a question of persons standing, so to say, outside the "normal variation," who for some quite peculiar reason give qualitatively different responses to the dissemination of BCG? I do not think we can answer this question today.

The important findings of Gormsen, however, indicating a variation in size and amount of the epithelioid granulomata from one individual to another, might perhaps point in the direction of the first possibility, and so may the few cases of benign hematogenous metastases, possibly due to BCG, which have been reported. Such cases may perhaps be more frequent than we assume today, even though the serious and fatal cases must in all probability be extremely rare.

The matter is certainly highly complex. For the time being we have ample material for speculation but very little basis for definite conclusions.

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# BCG AND VOLE VACCINATIONS

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## INTRODUCTION

I had hoped that the preliminary report of the Medical Research Council's BCG and Vole Trial on 50,000 school children would have been available in time for this Symposium.[2] As no such statistical results are yet available, I have changed the theme of my paper to a frank statement of our present position with all its unfinished investigations. By this means I hope that I may suggest certain lines of research to you and, in return, gain advice for myself from our subsequent discussions.

It may interest you to know that my first practical experience with BCG vaccination was in Park and Kereszturi's BCG clinic in New York some twenty-four years ago, when I was an intern in pediatrics at the Bellevue Hospital under Hendee Smith. On my return to England, I tried hard to get BCG introduced, but it was not until 1949 that it was first issued by the Ministry of Health to approved vaccinators for use in the three main exposed groups: nurses, medical students, and tuberculosis contacts. Under the National Health Service, England is divided administratively into thirteen regions; in 1950, I was appointed adviser in BCG vaccination to the Oxford Region which has a population of one and a half million. This brought me into contact with A. Q. Wells who was working in Oxford on his vole vaccine. In 1951, Wells and I organized a uniform method of recording all BCG and vole vaccinations in the Oxford Region, which is the basis of the comparative research on which we are at present engaged.

## OBSERVATIONS

### *The BCG Vaccination of Special Exposed Groups*

*Tuberculin testing:* The first essential was to formulate a standard technique throughout the Oxford Region. Knowing that the allergy produced by BCG vaccination is not strong and has a tendency to wane, I decided that the Mantoux 100 TU should be used at all times as the criterion of a negative reactor. Later, when the Tuberculosis Research Office of the World Health Organization published the result of its research on nonspecific reactions (1), I was told that by using so strong a test I might be excluding genuine negative reactors. I admit that this may be so in theory, but further research by the Tuberculosis Research Office has shown that nonspecific reactions are not common in England, Denmark, or your southern states (2). The practical disadvantage of using 100 TU before vaccination is that it requires a preliminary test, though it may be safely used alone for conversion testing.

The results up to the present have justified this decision. Of 5,838 patients who completed BCG vaccinations in the Oxford Region, 5,821 have converted

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[2] Has since appeared in *British Medical Journal*, 1956, No. 4964, 413.—*The Editor*.

their tuberculin reactions to positive (99.7 per cent). The 17 unconverted patients were immediately revaccinated and 14 then "converted"; this gives an over-all conversion rate of 99.9 per cent. Of the 3 persistent negative reactors, 2 were known cases of sarcoidosis and the third was shown to have a tuberculin-neutralizing factor in her blood by the method of Wells and Wylie (3).

*The reaction at the site of vaccination:* The fresh BCG vaccine issued by the Ministry is imported weekly from Copenhagen; it has a strength of 0.75 mg. per ml., and is given intracutaneously. Freeze-dried vaccine from Paris is held in reserve and used in emergencies. At the time of tuberculin conversion, in 99.0 per cent of the patients there was a papule at the site of vaccination; some degree of breakdown of the skin over the papules occurred in 72.3 per cent of the cases.

*Local abscesses:* A cold abscess at the site of vaccination appears to result from the vaccine's being injected subcutaneously. This usually happens with unskilled vaccinators, in struggling children, or in infants. Twelve cases have occurred in our series (0.2 per cent). Eight were in children less than two years of age; three of the others (and in one of the 8 children) occurred in a family of four vaccinated by a "locum tenens." The twelfth occurred in a sixteen-year-old child.

*Abscesses of lymph nodes:* Abscesses of lymph nodes occurred in 17 patients who were vaccinated with the Danish vaccine. Fourteen were in infants in their first year and the remaining three were in the age group one to four years; as nearly half of our vaccinations fall in the zero- to five-year-old age group, our over-all incidence (0.3 per cent) is disproportionately high.

It has been suggested that it is unwise to vaccinate infants on the leg at the age of diapers, when the inguinal lymph nodes already bear a heavy load. Of 1,247 infants less than twelve months who were vaccinated on the arm, 11 developed lymph node abscesses (0.9 per cent); of 55 vaccinated on the leg, 6 developed lymph node abscesses (11 per cent). This difference is highly significant ($p < 0.001$).

It has also been suggested that the Danish vaccine was, until recently, weaker during the summer months from the effects of sunlight during its preparation. Dividing the year at the equinoxes, six abscesses occurred with summer vaccine and eleven with winter vaccine. This difference is not significant ($\chi^2 = 0.27$, $n = 1, 0.50 > p > 0.70$).

*Tuberculosis after vaccination:* As we have no control group, it is impossible to say whether or not the persons vaccinated show evidence of protection. There have been no deaths from tuberculosis in this series and only 3 patients are reported to have evidence of a tuberculous "condition." The first was a thirteen-year-old contact who developed a pulmonary cavity two years after vaccination and from whom a virulent human strain was cultured. This is a genuine case of tuberculosis developing despite BCG vaccination. The second patient was a nurse who was found to be tuberculin negative, but was not vaccinated until five months later. Her vaccination showed an accelerated reaction starting on the fifth day. Three years later, a chest roentgenogram showed a left apical tuberculoma. The evidence suggests that she may have been infected before

vaccination. The third was an infant contact who was vaccinated at two months. At eighteen months, although there were no clinical signs or symptoms, this patient was reported to have a bilateral calcified complex roentgenographically. As the films were lost and the parents refused to bring the child back to the clinic, these findings were never confirmed.

*The Mass BCG Vaccination of Thirteen-Year-Old School Children*

In an attempt to reduce the relatively high tuberculosis morbidity rate of young adolescents in industry, the Ministry decided in 1953 to extend BCG vaccination to children leaving school. The thirteen-year-old age group was chosen so that the child could remain under observation before leaving school at fifteen. Up to this time our organization in the Oxford Region was geared to vaccinate approximately 1,500 persons a year. This new extension added some 25,000 children. It was clear that the utmost simplification of vaccination technique was necessary.

*The Heaf multiple-puncture tuberculin test:* The first requirement was a "one-shot" tuberculin test. The Mantoux 100 TU had to be discarded, and it was generally agreed that the 10 TU was the strongest we dared to use. An inevitable drop in the conversion rate was feared. In 1951, however, Heaf had introduced a new multiple-puncture tuberculin test which had been found safe to use as a one-shot test (4). It was thought to be as good as, if not better than, the Mantoux 10 TU and had the additional advantage that no skill was required in its use. The test is performed with a special gun which stabs six needles into the skin to a standard depth through a film of "adrenalinized" Old Tuberculin (OT)[1] or of PPD, 2 mg. per ml., to which glycerin has been added to give it the same consistency. The test is read after three days. Heaf classifies positive reactions as:

First degree: Four or more palpable papules.
Second degree: The papules have coalesced to form a ring of induration.
Third degree: A plateau of induration.
Fourth degree: A greater reaction (5).

*A comparison of the Heaf with the Mantoux test:* As little work had been done on the sensitivity of the Heaf test, I tried to calibrate it against the different strengths of Mantoux. Whenever I had to do a Mantoux test, I carried out a Heaf test on the opposite arm. As I was carrying out prevaccination, conversion, and follow-up tests, this was not a random sample. Nevertheless, I think that the results, which suggest that its sensitivity lies between that of the Mantoux 10 and 100 TU, indicate that a proper trial of the Heaf as a one-shot test is warranted. The results are shown in tables 1 and 2. At first sight, the fact that the Heaf appeared to give 14 "false positives" is disturbing, but on investigating these cases it was found that 13 of the patients had been vaccinated and had previously been positive to a Mantoux 100 TU. It is possible that the Heaf test elicited a true allergic response although the Mantoux had failed.

For some of these Heaf tests I used "adrenalized" OT and for the others PPD. A preliminary analysis which was completed just before I left England shows that "adrenalized" OT gave significantly more consistent results than PPD with respect to the Mantoux test.

[1] Old Tuberculin to each milliliter of which has been added 1 drop of 1 in 100 epinephrine.

TABLE 1

THE HEAF TEST COMPARED WITH THE MANTOUX TEST

| Mantoux | Heaf | |
|---|---|---|
| Positive | Positive | Negative |
| 1 TU – 304 | 302 (99%) | 2 |
| 10 TU – 139 | 136 (98%) | 3 |
| 100 TU – 354 | 279 (79%) | 75 |
| Negative | Negative | Positive |
| 1 TU – 373 | 137 | 236 |
| 10 TU – 5 | 3 | 2 |
| 100 TU – 260 | 246 (95%) | 14* |

* Thirteen of these subjects had been vaccinated.

TABLE 2

A SUMMARY ANALYSIS OF HEAF AND MANTOUX RESULTS

| Diameter in Mm. | Heaf Reading | | | | | |
|---|---|---|---|---|---|---|
| | 0 | I | II | III | IV | Total |
| **Mantoux 100 TU** | | | | | | |
| 0 | 237 | 8 | 5 | 1 | — | 251 |
| 1– | 9 | — | — | — | — | 9 |
| 6– | 13 | 14 | 8 | 6 | — | 41 |
| 11– | 24 | 24 | 25 | 29 | — | 102 |
| 16– | 24 | 34 | 37 | 27 | — | 122 |
| 21+ | 14 | 26 | 25 | 26 | — | 89 |
| **Total** | 321 | 106 | 100 | 87 | — | 614 |
| **Mantoux 10 TU** | | | | | | |
| 0 | 3 | — | — | 2 | — | 5 |
| 1– | — | — | — | — | — | — |
| 6– | 2 | 7 | 7 | 4 | 1 | 21 |
| 11– | 1 | 4 | 17 | 39 | — | 61 |
| 16– | — | 5 | 16 | 28 | — | 49 |
| 21+ | — | 1 | — | 6 | 1 | 8 |
| **Total** | 6 | 17 | 40 | 79 | 2 | 144 |
| **Mantoux 1 TU** | | | | | | |
| 0 | 136 | 38 | 42 | 42 | — | 258 |
| 1– | 1 | 17 | 22 | 67 | 8 | 115 |
| 6– | 2 | 8 | 26 | 127 | 11 | 174 |
| 11– | — | 2 | 8 | 61 | 4 | 75 |
| 16– | — | 6 | 3 | 27 | 5 | 41 |
| 21+ | — | 1 | 2 | 11 | — | 14 |
| **Total** | 139 | 72 | 103 | 335 | 28 | 677 |

During this pilot survey, I noticed that the cutaneous reaction had only begun to fade after seven days. The country doctor has long sought a test which could be given at one weekly clinic and read at the next, so I carried out a second survey in which I read the Heaf test after seven days. This proved rather disappointing. A similar trial by the Army, however, found that the readings were better at seven days than at three (6). Here, I suggest, is a further piece of research that should be done.

*Results:* Shortly before I left, the results of the vaccination of our first batches of school children were coming in. Unfortunately, as might have been expected from an expansion which involved many doctors who were becoming vaccinators for the first time, some of these results proved more of a lesson in administration than a contribution to scientific knowledge. Of 1,543 vaccinated school children, 1,484 converted to a Mantoux 10 TU; this gives a conversion rate of 96.2 per cent. Of another group of 304 school children, 298 converted to the Heaf test; this gives a conversion rate of 98.0 per cent. As was expected, the conversion rates are not so good as those with the 100 TU. By the New Year our growing pains should be over and I hope that our later figures may be of considerable interest.

## *Vole Vaccination*

*The history of the vole vaccine:* In 1937, A. Q. Wells, who was working on epizootic disease in the vole, discovered that some of these animals were dying from tuberculosis. On typing the strain he was surprised to find that it failed to produce progressive disease in any laboratory animal, even the guinea pig, unless injected in massive doses. The inoculation was followed only by the appearance of a local lesion which developed for four to six weeks and then regressed. As much as 5 mg. moist weight of culture could be injected subcutaneously into a guinea pig with subsequent complete resolution, while $10^{-7}$ mg. inoculated into a vole produced progressive disease and death. This fact, together with certain morphologic and cultural differences, seemed to justify its classification as a new type of tubercle bacillus. Wells (7), therefore, called it "the murine type of tubercle bacillus." Today it is usually referred to simply as "the vole bacillus."

*Animal experiments:* Realizing the similarity of the behavior of the vole bacillus to BCG, Wells studied its protective effect on guinea pigs. In 1952 Wells and Wylie found that guinea pigs vaccinated with the vole bacillus survived 163 days after a challenging infection, while BCG-vaccinated guinea pigs survived only 106 days; the control animals survived only 53 days. They also found that, whereas the resistance to infection after BCG dropped as the tuberculin sensitivity faded, with vole vaccination the degree of protection remained unchanged for six months after disappearance of the tuberculin-sensitive state.

*Vole vaccination trials:* After five years of intensive experimental work on animals, Wells started human vaccination in 1942. He and his colleagues have now vaccinated more than 2,500 persons. Many more have been vaccinated in the trials of the Medical Research Council in England and of the Tuberculosis Research Association in Africa, which are still in progress.

*The safety of vole vaccination:* No generalized disease following vole vaccination has yet occurred. It has become apparent that the vole vaccine is a much more powerful antigen than BCG and the dose now given is correspondingly smaller. Whereas the intracutaneous dose of BCG may be 0.1 mg., the optimal dose with vole is only 0.0001 mg., that is, only a thousandth of the BCG strength. In the early days before this was realized, a dose of 0.1 mg. of vole was given in several cases. Although a thousand times the optimal dose, it produced no more than a rather violent local reaction.

*The effectiveness of vole vaccination:* Among the 2,500 persons, consisting mainly of mental defectives in institutions, who were vaccinated by Wells and his colleagues, only two cases of tuberculosis have occurred. One was a transient case of tuberculous endometritis and the other was a patient with pulmonary tuberculosis who has since recovered. From both of these patients, human tubercle bacilli were isolated. A third patient who is not included was infected just before vaccination and died six months later; an accelerated reaction reaching a maximum in ten days revealed a preallergic state. The incidence of tuberculosis in this group was 0.08 per cent.

A control group of 717 negative reactors to a Mantoux 100 TU of the same age and sex was observed in the same institutions. During the same period, 17 of these negative reactors developed tuberculosis, 4 of whom have died. Human tubercle bacilli were isolated in each case. The incidence of tuberculosis in this group was 2.37 per cent, which would appear high were it not that these were mental defectives and the figures cover a period of years. The incidence of tuberculosis in the vaccinated group was therefore about one-thirtieth that of the controls (9).

*The development of vole vaccination:* Subcutaneous vaccination was tried originally, but was soon abandoned on account of the severe local ulceration it produced. Multiple-puncture with 2 mg. per ml. was successfully used on most of this series and produced a conversion rate of 99.9 per cent to a final Mantoux 100 TU, although the great majority of those vaccinated reacted to 1 TU; the two who were persistent negative reactors were shown to have a tuberculin-neutralizing factor in their blood. Recently intracutaneous vaccination with vole vaccine has become increasingly popular.

In some cases a lupus-like reaction occurred over the multiple-puncture site. As these lupoid reactions were always observed in low-grade mental defectives who scratched their vaccinations with feces-contaminated hands, no great importance was attached to them. Recently, however, it has been found that, among a group of normal boys, 5 per cent of those who were vole-vaccinated by multiple puncture developed lupoid reactions at the end of the first year, and 15 per cent at the end of the second. This did not occur in any of those vaccinated intracutaneously (10).

As a result of this, multiple-puncture vaccination with the vole bacillus has been largely abandoned and intracutaneous vole vaccination substituted. So far no lupoid reactions have been reported by this method. Unfortunately, there is a much greater tendency for intracutaneous vole bacillus vaccinations to produce

lymph node abscesses; this may be due to clumping of the bacilli in the vaccine. Wells is trying to overcome it by centrifuging and preparing the vaccine until it is clear of all clumps, and then standardizing it by a count. A dose of 50,000 bacilli is at present under trial.

Another idea that is also under trial at this moment is an attempt to protect the lymph nodes by fixing the bacilli at the site of vaccination. In a recent experiment on guinea pigs, Vollum found that, if dead vole bacilli were injected intracutaneously four weeks before vaccination, there was a marked check in the spread of the live bacilli from the skin to the lymph nodes (11). Wells and I are conducting a trial in two institutions, in one of which a dead bacillus inoculation was given four weeks before vaccination. The local reactions at four weeks were similar in both institutions, except that I noticed an area of induration in the skin below the papule in five of the subjects who had previously been given dead bacilli. Later two of these areas of induration broke down to uncomfortably large ulcers, which suggests that localization had, indeed, taken place. No one in either group has developed a lymph node abscess up to the present. A further reduction in the dosage of live vole vaccine may overcome these fierce local reactions.

Meanwhile Šula has just reported that more than 11,000 infants have been vaccinated with vole bacillus in Prague, two intracutaneous doses of 0.25 mg. being used. He finds that the local reaction is smaller than with BCG, that the allergy produced is stronger and that, unlike BCG, no adverse effects of vaccination have been observed (12). The milder reaction obtained by Šula may in part be due to the fact that the virulence of the Wells strain has been constantly maintained by animal passage. In contrast, Šula is working with strain OV166 which was isolated from a vole in 1947 and appears to have been grown on artificial media ever since. As a result some attenuation of Šula's strain may have occurred.

### *A Comparison of BCG and Vole Vaccination*

BCG is a well-tried and accepted vaccine, while vaccination with the vole bacillus is still suffering from growing pains. Nevertheless, I think there are two factors which make a continued study of the vole vaccine important, its superior antigenicity and its stability. It is now generally accepted that there is a considerable variation between the BCG vaccines produced by different centers; as there is no animal for which BCG is pathogenic, this cannot be corrected by animal passage. Vole vaccine can be standardized at any time in any place by passage through a vole; in practice this is done by Wells at every tenth subculture. It is of interest to note that in guinea pigs not only does the allergy produced by vole vaccination last longer than that produced by BCG, but resistance continues after tuberculin allergy has gone. Whether this occurs in man remains to be seen. The results of the two trials already mentioned should throw further light on the comparative value of the two vaccines.

*Acknowledgments*

In conclusion I should like to thank the Oxford Regional Hospital Board and the Medical Research Council for the grants which have made this work possible. In particular I

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## GENERAL DISCUSSION

Dr. Dubos (New York, New York): I really want to speak only about Dr. Ström's work. Everything I might say will be so highly complimentary that it won't fit in with Dr. Waring's admonition that we should try to stir up ill feeling if possible. So, if you don't mind, I will start in a somewhat different vein.

On two occasions, Dr. Irvine, you made statements that I think are unjustified and incompatible. They occurred within about half a minute of each other. In one sentence you recited to us that Šula had been doing extensive vole vaccinations in Prague, and you explained away the difference in results that he obtained as against yours by the fact that he was using a strain of vole bacillus which probably had changed because, since 1947, it had been maintained in artificial culture media. Well, I believe that.

Then, about one and a half minutes later, you said that one of the great advantages of the vole bacillus is that it does not change—that it is much more stable than BCG. After that you corrected yourself by saying that it is stable because you can maintain it by repeated passage in voles. Then you made another statement that is false. You said this is quite different from the situation with BCG because BCG is not pathogenic for animals.

I think that is not correct. BCG multiplies in any animal into which it is introduced, and the rate of its multiplication is an expression of its pathogenicity. I think the same funda-

mental difficulty exists with reference to the vole vaccine that exists with reference to BCG; namely, that they are changeable organisms. They change when being maintained *in vitro*, and probably they also change even more *in vivo* than *in vitro* because it is a little easier to standardize *in vivo* conditions than *in vitro* conditions. Let that be enough to create some ill feeling.

I also had something unpleasant to say about Dr. Ström, but I am afraid I have forgotten it. I hope it will come back to me.

May I express my personal opinion that the discoveries made by Dr. Ström, using tubercle bacilli or BCG, that have incorporated radioactive phosphorus constitute, I believe, the most original contribution to the problem of immunity to tuberculosis for the last twenty years. So, if you don't mind, I shall very briefly restate some of his findings for those of you who have not had the privilege of watching Dr. Ström's work for the past few years.

What Dr. Ström does is to grow BCG in the presence of $P^{32}$, permitting the radioactive phosphorus to be incorporated into the body of the bacillus. That permits him to trace with a Geiger counter the travels of the BCG bacilli, the dispersion of the BCG bacilli in the organs of the animal host.

I shall single out only two of the discoveries that he reported this morning. One permits him to show that virulent tubercle bacilli injected into an animal which has previously been vaccinated do not become distributed as rapidly and as widely in the organs of the host as do the same bacilli injected into a normal, nonvaccinated animal.

So, I believe we have completely objective proof that vaccination does minimize, does retard, the spread of the bacilli from the point of injection.

The second discovery—which I am very surprised has not been exploited; there should be 100 workers studying this problem—is that, if you follow the rate of urinary excretion of radioactive phosphorus from marked bacilli that have been injected into a normal animal and compare it with the rate of excretion in the urine of an animal that has been vaccinated, you will observe within a very short time—How soon—within an hour?

DR. STRÖM (Karolinska Sjukhuset, Sweden): One to two hours.

DR. DUBOS: You will observe a much larger amount of $P^{32}$ being excreted in the urine of the animal that has been vaccinated.

Since most certainly the $P^{32}$ has been released from the body of the bacilli, this is the most compelling evidence—in fact, absolute evidence—that the vaccinated animal has the capacity to destroy, within an hour after injection, many of the bacilli of superinfection.

In my opinion this experiment is so crucial that it demonstrates once and for all, without any need of argument or other demonstration, that vaccination endows the vaccinated animal with the ability to destroy the bacilli and to destroy them very rapidly.

Second, I have no doubt that this discovery is the key that will permit us to analyze the mechanisms whereby the vaccinated individual has acquired these bactericidal properties for bacilli of superinfection.

Dr. Ström indicated this morning that his findings are being developed and extended in Montreal. I have been given the privilege of reporting to you, as Dr. Ström knows, that this discovery is also being extended in Paris at the International Children's Center, using $C^{14}$ instead of $P^{32}$ to produce radioactive bacilli. This work has been done in Paris by Dr. Pasquier. I happen to know that there are some conflicts of opinion on certain technical details between Dr. Ström and Dr. Pasquier, but I think they are only of importance to them because they are competitors in the same field. They are somewhat minor to those of us who are looking for a technique to demonstrate, in an objective manner, evidence of bactericidal power following vaccination.

I should like to take a few more minutes to agitate the problem which Dr. Irvine alluded to on several occasions, namely, the differences between different samples of BCG vaccine preparations. I am one of those who is convinced, as you well know, that there are profound differences which are dependent upon intrinsic characteristics of the different strains of BCG that are used the world over in the preparation of the vaccine.

There are other differences equally important, however, which come from the techniques

of preparation of the vaccine; and I might call to your attention, for those of you who have not seen it, a recent issue of the *Revue d'Immunologie*, in which are reviewed all of the studies that have been carried out in Paris to express in an objective manner the qualitative and quantitative differences between the various preparations of vaccine.

I do not intend to review these findings, but let me single out one or two of them which are of such enormous practical importance that I have no doubt that they should be taken into consideration in the future in the preparation of the vaccine.

Differences resulting from desiccation: I think it has been the observation of practically everybody—and it has been confirmed in Paris—that desiccated vaccine, even with the same number of living cells, always is somewhat less active than fresh vaccine. A valuable characteristic of these studies is that they attempt to integrate within one organized set of studies results on the same batch and the same ampoule of vaccine tested both *in vitro* and in animals, mice and guinea pigs, and in human beings. Using these four techniques, they have clearly demonstrated that nobody has yet worked out a reliable technique of desiccation.

The difference in activity expresses itself by a slower rate of multiplication in any kind of culture media, egg or Tween®-albumin media, by a much slower rate of multiplication in the animal; and in man, by slight but significant differences in the degree of allergy which is elicited.

The only other point that I shall single out, and which is perhaps the most important, is the enormous difference in biologic activity of the vaccine, depending upon the age of the culture from which the vaccine is prepared.

As you know, in the past it has been a tendency (and it still often is a tendency) to let the culture grow for as long as possible so as to obtain larger yields of bacilli and of vaccine. In reality, however, it has now been convincingly shown by the techniques I mentioned—also by the rate of incorporation of $P^{32}$ and $C^{14}$ by the bacilli—that the younger the culture the more competent it is to multiply *in vivo*, the more rapidly it multiplies *in vitro*, the more efficiently it binds $P^{32}$ or $C^{14}$, and the more active it is in human beings.

So, there is no doubt that in the future it will be thought advisable to sacrifice yields in terms of milligrams of bacilli in an attempt to obtain cultures of greater activity for the preparation of vaccine. That will be done by using younger cultures.

Professor Debré has informed me that he has brought with him a dozen or so copies of this issue of the *Revue d'Immunologie* and that he will be willing to distribute them to any of you who may be interested.

DR. WARING (Denver, Colorado): I have a couple of questions I would like to ask. One is based on an experience of a former resident at the Colorado General Hospital. He came to me with this statement:

> I have twice been vaccinated with BCG and still have a negative tuberculin test. I have passed through my service at the Colorado General Hospital on the tuberculosis ward. I have passed through a service at the National Jewish Hospital. I have passed through a service at the Fitzsimons Army Hospital on the tuberculosis ward, and I still have a negative tuberculin skin test. Should I be worried? Is my resistance good or bad? How can I find out?

The second question is this: Are we justified in thinking that tubercle bacilli, inhaled naturally by human beings, disseminate as rapidly and as thoroughly throughout the body as tubercle bacilli are spread after a subcutaneous inoculation in animals?

A third question: Perhaps some light might be thrown on this by Dr. Robinson from his work at the National Jewish Hospital. After reversion of the tuberculin test under chemotherapy in children who formerly have had a positive tuberculin test but negative X-ray pictures, is acquired resistance lost? How can you measure whether there is more resistance than if they had never had a positive tuberculin test or had never been treated?

Those problems are very simple, very clinical, and elementary.

DR. KOCH-WESER (Chicago, Illinois): Since Dr. Dubos said somebody else should have done some of these experiments with labeled bacilli, I think I should report that we did some of this work, and it was reported in the Atoms for Peace Conference proceedings of last August.

Our group at the University of Chicago labeled tubercle bacilli of an avirulent strain (R1Rv) with $C^{14}$ labeled methyl acetate. We found one difficulty. As long as those bacilli were alive, they turned over the acetate quite rapidly in the medium, and presumably also in the organism. Therefore, we used for that particular study pasteurized tubercle bacilli which we found had retained the antigenic activity, and we injected bacilli with rather high radioactive counts in the feet of guinea pigs which previously either had tuberculosis or were vaccinated, and of guinea pigs which were tuberculin skin test negative. We found results which confirmed Dr. Ström's results; namely, the guinea pigs which previously had been vaccinated retained the radioactivity for a much longer time in the foot. Also the radioactivity appeared in the regional lymph nodes, which we took out, to a much less degree than in those guinea pigs which had not been vaccinated. In the latter, the radioactivity disappeared quite rapidly from the foot and appeared in the regional lymph nodes.

With living tubercle bacilli it is difficult to make any differentiation between the distribution of the radioactively labeled bacilli from the point at which you inject them and breakdown products from the tubercle bacillus which are distributed in the organism.

I would also like to ask one clinical question. One of the main arguments we always hear against BCG is the argument that the positive tuberculin skin test loses its value as a diagnostic test. As an example, we encounter quite some opposition to giving BCG to our medical students, because the last two classes entered the school with less than 20 per cent positive skin tests. More than 80 per cent had negative tuberculin skin tests. It was believed by the student health authorities that it was more worth while to skin test those students every year, or possibly every six months, and retain the diagnostic value of the converter, than to spoil this by giving BCG.

Dr. Waring: What has always worried me about the tuberculin test and its relation to resistance is that we always seem to be agreed that tuberculin allergy and resistance are two entirely separate things, and yet with BCG vaccination we use the tuberculin reaction as an index of resistance.

Dr. Dubos, were you able to follow the question that was fired at you? It was leveled at Dr. Ström? Then we will call for more questions before you answer, Doctor.

Dr. Roger Mitchell (Denver, Colorado): I would like to ask Dr. Irvine whether he can read the Heaf test at two days or whether it really requires three days at the earliest.

I would like to ask Dr. Ström whether he has given labeled bacilli to animals receiving isoniazid and, if so, what happens to the bacilli.

Dr. Waring: Dr. Ström, does the presence of radioactive material in the urine indicate that the bacilli have been destroyed or are being destroyed?

Dr. Ström: Yes.

Dr. McDermott: I would like to ask another question of Dr. Ustvedt. That is, whether the four patients, at least one of whom grew to the age of nineteen years, if I remember correctly, had any unusual history with respect to boils, furuncles, measles, repeated respiratory infections, or any other clinical manifestations of repeated infection.

Dr. Robinson (Denver, Colorado): Along with Dr. McDermott's question, we all know that, of the many, many children who are vaccinated against smallpox, there is a small number in whom the smallpox vaccination spreads and progresses until the child dies. I wonder if there is a history in any of these four patients of smallpox vaccination that was successful.

I would also like to ask Dr. Ström how many cases of tuberculous meningitis he knows of which occurred in infants who had been properly vaccinated with BCG.

Finally, I might take this opportunity of answering your question, Dr. Waring. I don't know the answer, but I would wonder if giving these children (in whom the tuberculin test has been reverted to negative) labeled BCG might let us find out whether or not they were immune.

Dr. Waring: Did you do any tests along that line or any other line to determine whether there was a residual resistance of some sort? (No)

I might ask the experts: Do they have any good practical method of measuring resistance other than this tuberculin test that we use as an index of resistance? (No)

Are there other questions?

DR. WILLIAM STEAD (Minneapolis, Minnesota): I have noted—in Dr. Wallgren's reports, I believe—that the BCG-vaccinated individual gives a different kind of positive tuberculin reaction. In line with Dr. Koch-Weser's question, I wonder whether the experts can tell the difference. In Minnesota I haven't seen enough people vaccinated with BCG to be able to see these two kinds of reaction.

DR. CANETTI: I have the feeling that Dr. Ström has been a little severe on oral vaccination with very high dosages of BCG, and so I want to ask Dr. Ström two questions.

First, on what grounds does he make the statement that in his opinion that type of vaccination does not lead to a good immunity? I think Dr. Ström said that in such a vaccination penetration of the vaccine occurs through the mucosa of the mouth.

Second, I would ask him if he has controlled this through any method with radioactive BCG or by any other means in human beings.

DR. WARING: Dr. Ström, I would like to ask: If you give this vaccine by mouth and if the child should cough or vomit, is there any risk of BCG vaccination otitis media?

DR. MITCHISON (London, England): I would like to ask Dr. Ustvedt if he has any information on the location of the tubercle bacilli in the lesions of the fatal cases of BCG infection; that is, whether these bacilli were intra- or extracellular in view of the failure of response to streptomycin treatment in at least one patient.

DR. CARROLL PALMER (Washington, D. C.): I have only one point, and that is whether or not you can use the tuberculin test for diagnostic epidemiologic purposes after BCG. It is important, and I think it depends entirely on what BCG you use.

Certainly we have had (particularly in the WHO Copenhagen office) a good deal of experience that would lead us to believe that, if you use a good, potent BCG, you absolutely cannot differentiate between a natural positive reaction from infection and a BCG vaccination.

BCG is a highly variable substance. I also know from our own experience that using weaker vaccines gives you less allergy, and you have some possibility of telling the difference. However, I think a good deal of confusion under the very best of circumstances will arise if you try to use the tuberculin test for diagnostic and epidemiologic purposes after BCG.

DR. JOSEPH GORDON (Albuquerque, New Mexico): I am formerly of Raybrook, New York. Since I know you like to have confusion confounded, I should like to relate an experience we had while I was at Raybrook studying, in guinea pigs, the drainage of the breast through its regional and local lymph nodes.

When silica was injected locally, apparently blocking the local draining lymph nodes (following which thorium was used), the local draining lymph nodes were by-passed and the regional lymph nodes were demonstrated. I wonder if that may have some relationship to Dr. Ström's work in the speed with which a previously infected local lymph node may have been partially blocked and whether your radioactive material is more rapidly picked up in regional lymph nodes and presented in the urine in vaccinated subjects.

DR. SCHMIDT: I would like to ask Dr. Ström whether the location of the phosphate in the turbercle bacillus has yet been defined. Also, what is the nature of the phosphorus-containing compound which is eliminated in the urine? And has it been identified as the same chemical entity both in the immunized and in the nonimmunized individual?

DR. WARING: I would like to have some questions from the heart of the meeting, the distinguished doctors on either side of the table, west and east.

DR. PLATOU (New Orleans, Louisiana): May I ask Dr. Ström if he has had the opportunity to measure excretion of $P^{32}$ from tagged BCG in patients with known, active tuberculosis.

DR. DUBOS: The unpleasant thing I had to say about Dr. Ström has come back to mind, but I would like to introduce it a little later when Dr. Ström has dealt with the many questions that he has already been asked.

DR. GIRDANY: The question of the relationship between allergy and immunity in tuberculosis is one that has been mentioned and will continue to be mentioned until better means are available of determining whether or not a person can have immunity without allergy. I think it becomes of practical importance in measuring the success of the BCG inoculation. The question I have is many-sided. First, I would like to know whether those patients who have had BCG inoculations, and who have developed tuberculosis subsequently, have had a markedly positive tuberculin test or whether such cases have been among those subjects requiring a lot of antigen to produce a reaction.

Second, is there any relationship between the severity of the local reaction and the amount of immunity? Perhaps we are wrong in assuming that there is such a relationship; but occasionally we have children from the Well Baby Clinic who come to us with lesions in their axillae that are quite large, and we tell the mothers that that is good as long as it does not break down, because presumably they are getting a good reaction and they are going to have good immunity. Is that valid?

DR. DUBOS: The question I meant to ask Dr. Ström comes up again with reference to the remarks made by Dr. Girdany. It comes from the misuse of a word, and this time it is not mere semantics—it is just complete immunological confusion about the most important aspect of the response of the body to tuberculosis.

I shall begin with Dr. Ström's statement this morning, and, as you will see, it applies exactly to the question that has just been asked.

Dr. Ström told us that he has seen vaccinated animals which had failed to give evidence of allergy which handled the tagged bacilli just as the immune animals did, and he suggested that as evidence that one could be immune without being allergic. The question has now come up again.

In reality, this is a most unfortunate misuse of the word "allergy." As you know, the word "allergy" was introduced by Von Pirquet and Dr. Schick (who is in this room) to mean something very clear, namely, the changed response of the body to any substance, living or dead.

In the case of tuberculous infection, allergy can express itself in part as one of its many manifestations by a change of response to tuberculoprotein, but allergy can also be present to polysaccharide, possibly to nucleic acid, probably to every chemical constituent of the tubercle bacillus.

To speak of allergy only when one means hypersensitive response to tuberculoprotein is really misunderstanding or forgetting the complexity of the total response of the body to infection. Even then, the use of the tuberculin test cannot have a very simple and limited interpretation, even when one uses PPD. Please remember what "PPD" means. It does not mean "pure protein derivative"—it means "purified," which is very different from "pure."

I need not make a statement which Dr. Long and so many others could document much better than I; but even the most purified preparation of PPD contains not only proteins or breakdown products of proteins, but many different proteins; it contains some nucleic acid; it contains some polysaccharides and, when you measure the response to either Old Tuberculin or PPD, you really measure the summation of several very different types of immunologic phenomena.

In addition, there are certainly many such responses which do not express themselves in the skin test. That is known throughout the fields of bacteriology and immunology. For all we know, it is precisely one of those for which we have not yet obtained a convenient serologic test which is responsible for resistance or immunity. That would be the one to give us the significant answer to the question that you ask; namely, how can we decide whether a person is resistant or not?

So, I wish that in the course of our discussion we would not use the word "allergy" except in the original sense, meaning a changed reaction to all the components of the bacilli. If we want to speak of the tuberculin test, let's have in mind clearly that we are talking about the crudest possible sum of reactions to a multiplicity of different components, for each one of which there may be different and variable levels of immunologic response.

For example, there is no doubt but that part of the skin response that is observed is one that is not due to the protein component but one which is due to the polysaccharide. It is not possible to include or use the skin test as a precise measure of allergy. So, Dr. Ström, I do not think you should say that the animals were not "allergic." You can only say that they did not respond to those components that happened to be present in the preparations of PPD that were used.

Dr. Middlebrook: Dr. Dubos, I think you were a little cavalier in the expression of your concept of pathogenicity. In recent studies by air-borne infection methods, compared to subcutaneous or intracutaneous challenge methods, with strains of tubercle bacilli which have different properties, we have been struck by what I think might fairly be called a differentiation between two facets of potency of microorganisms—of tubercle bacilli—that should be more clearly differentiated. One property may be described by a term already in common use, "infectivity"; and the other by "pathogenicity." I will describe a little more precisely what I mean.

If any of the isoniazid-resistant mutants of ordinary strains of tubercle bacilli which are catalase negative are used to challenge guinea pigs intracutaneously, and are compared with BCG inoculated by the same route, it would seem that they differ very little in this particular host. One can hardly distinguish any clear difference between them.

On the other hand, if one exposes guinea pigs to those two different strains, a BCG and a catalase-negative strain, by the air-borne infection route, one sees a marked difference between them in the following sense: The catalase-negative mutants regularly produce grossly visible pulmonary lesions with central necrosis, whereas BCG at no time in our experience thus far (and we haven't kept the animals very long; really not more than a few months) manifests any gross or even microscopic lesions that we can demonstrate after inhalation of the same number of living bacterial cells. These experiments illustrate the marked differences in infectivity between strains of almost equal pathogenicity.

I wonder if this orientation, this differentiation between what might be called the ability to multiply at first by one route compared with another route and the ability to continue to multiply after a certain type or level of host response has occurred, might not be profitably applied to many of the problems of vaccination.

Dr. Waring: I am afraid time is going on, and that we ought to give this morning's speakers an opportunity to reply. Dr. Ström, would you answer the questions that have been fired at you?

Dr. Ström: I will try to, although I haven't caught all the names.

First, Dr. Waring's questions about the persons who are BCG vaccinated once, twice, perhaps several times, and fail to become tuberculin positive.

As I said previously, those people handle the labeled BCG exactly like the people who are BCG vaccinated and tuberculin positive. I have taken this as a kind of proof that these tests might actually be tests of immunity.

You asked, too, if one could extend conclusions from experiments with animals to human beings.

I haven't published any results on this possibility, but I think there are several lines of evidence indicating that the dissemination in human beings has been quite the same as in animals. I haven't been able, of course, to do a critical comparison between immunized and nonimmunized children.

Another question was: If a previously positive tuberculin test reverted to negative, does this mean a loss of immunity?

I can tell you that last year we had the opportunity to examine about 53 cases of lymphogranulomatosis which we followed for a time. When the patients reverted from tuberculin positive to negative, according to the course of the illness, the tagged BCG behaved quite independently of the skin test. That is to say, as far as I can see, the immunity was intact, unchanged, while the reaction of the skin tuberculin test was changed.

Dr. Koch-Weser, I was very glad to hear of your work in this field. I didn't know about it. I should have, of course. There are I suppose, many people working with labeled

bacilli and unlabeled bacilli at present, not only in Stockholm, Montreal, and Paris. I know there are some German workers, too. You asked about turnover of $P^{32}$ from bacilli to media. Of course, that has offered the greatest technical difficulties. We have tried in different ways to wash the bacilli. We used a kind of diffusion first to let the bacilli pass the skin, but it was very difficult to handle bacilli afterwards.

Then we have washed them with phosphate solution of a certain pH. We always wash the bacilli very thoroughly three or four times.

Then the question is: How stable is this label? You know that, of all the mineral elements composing the tubercle bacilli, about 50, 51, or 52 per cent, or something like that, is phosphorus. That is why we have chosen phosphorus to label. A study of tubercle bacilli in Paris showed clear evidence that the content of $P^{32}$ is left untouched in the body of the bacilli for at least two or three days on transfer to a new culture medium without $P^{32}$.

The bacilli constantly lose a certain degree of radioactivity, as you have probably observed. We calculate about 5 per cent every time the bacilli are washed. You will also find about 5 per cent of the radioactivity in the rinsing water. That is the nearest we have come to meeting this problem. Of course, there must be a certain exchange of phosphorus between the tissues and the bacilli; but this is why we have only interpreted the examinations made for a few hours after animal or human inoculation.

We have not yet used this technique in drug-treated animals. I want to say to Dr. Dubos, in response to his kind words in the beginning, that we are a team in Stockholm; and one of my colleagues, Dr. Widström, is at present working with antimicrobial drugs in treated animals.

Dr. Robinson, I will answer your question. We haven't observed any case of meningitis or post-primary tuberculosis in BCG-vaccinated children in Sweden. This is one of the facts that Dr. Wallgren is always very keen to stress when describing the results of BCG vaccination. We have had, I should think, only 14 or 15 cases of miliary tuberculosis or meningitis in Sweden during the last eight or nine years. That's all. In the last two years in the pediatric clinic in Stockholm we have had no case.

Dr. Canetti, your question is a very challenging one. I should have documented my claim, speaking of the oral vaccination, and I should have said we have worked with this problem for about two years. We are continuing but, to date, as I stated (I think I dare to say) that, when we first worked with this labeled vaccine, we studied the dispersion after the oral administration. This was done in animals, and we have tried to give labeled vaccine in different ways by mouth.

We have first starved the animals and then given them the vaccine with some carrot juice by mouth, and they have actually swallowed it. We have also tried to put a little catheter in the mouth to get it directly into the stomach, and in those two groups there was a difference.

In the group in which we introduced vaccine directly into the stomach, we obtained a very poor dissemination of the vaccine. Most of it was eliminated, not in the urine, but in the stools. On the other hand, in the group in which we gave the vaccine in the mouth, directly, the mucous membrane of the mouth showed a very high activity and the dissemination in these instances was proportional to the amount which had been absorbed by the oral mucous membrane. When we got absorption in the mouth, we got a better, a higher resistance against tuberculosis than when we put it directly in the stomach. Still, there was a difference between the elimination of labeled phosphorus in the urine in the animals so vaccinated. Most of the animals behaved more like the nonvaccinated animals. Also, when we exposed the orally vaccinated groups to tuberculosis, we got about the same amount of tuberculosis as in the controls. So, as far as I can see, it really seems not to give much immunity.

I plan to publish those results. I realize very well that they must be quite well documented. In any case, I feel certain of what I am saying when I give these data.

Dr. Schmidt asked me about the nature of the phosphate. You see, if you use labeled phosphate, radioactive phosphate, the actually labeled dose is nearly negligible chemically.

I mean, you can't measure the phosphate in the urine as phosphate. It is just the radioactivity that is measured.

In some experiments of Dr. Sternberg in Montreal, he has found (using much larger quantities of labeled acetate) sodium acetate in the urine.

Dr. Platou asked me if we had applied this technique in the actively tuberculous. Yes, we have, and they act quite like the previously vaccinated persons with no previous natural tuberculous infection. The only complication in such cases is the very strong local reaction with necrosis.

Now, I come to the last questions, the unpleasant ones. First, in our experimental studies the tuberculin reaction is not always correlated with the degree of immunity: some persons who are supposed to have high immunity, or who have had a recent tuberculous infection, do indeed react regularly to the smaller doses of tuberculin. We know that. On the other hand, we have encountered subjects who never get a positive tuberculin skin reaction after BCG vaccination.

I will say then, in answer to Dr. Dubos, that first I have studied—or, rather, I haven't had any vaccinated animals which have not been tuberculin positive. Sometimes it is rather difficult to get them positive, but all animals I have used have been positive. The degree of the reaction, or the lack of reaction, in the skin I have only studied in human beings.

I should like to add that we have prepared a $P^{32}$ labeled PPD tuberculin. This was applied to guinea pigs by intracutaneous injections in quantity corresponding to 10 milligrams of Old Tuberculin. This was a preparation from a labeled vaccine. The radioactivity disappeared very rapidly from the local site of injection as does ordinary labeled phosphate solution, and we couldn't see any difference between tuberculous and nontuberculous animals.

I hope I have answered all the questions.

Dr. Waring: Dr. Ustvedt, would you like to answer the questions directed to you?

Dr. Ustvedt (Oslo, Norway): I can be very brief. Dr. McDermott asked what infections these patients previously had had, particularly the nineteen-year-old man. He had had the ordinary childhood diseases which we encounter. I mean measles and German measles, perhaps whooping cough. Nothing was reported as unusual with respect to these diseases. He had had acute appendicitis, as many people have. He had sinusitis, as many people have, and pneumonia. I don't know what you would say. It depends upon how much or how many infections you allow a single individual normally to have. I don't think one can draw any conclusions from his accumulation of infections.

The only peculiar point is that he did have in between these clinical episodes of illness a sedimentation rate of about 40 millimeters, which points to a certain lack of balance with regard to his proteins, or what have you. This was somewhat peculiar, at any rate, but we cannot interpret its importance.

Dr. Mitchison asked whether the bacilli in these cases were found intracellularly or extracellularly. In two publicatons it was mentioned that they were found both intra- and extracellularly.

Finally, Dr. Stead and others asked whether we are entitled to draw any conclusion with regard to immunity from the extent of the local reaction, and this I can answer according to the investigation of Dr. Palmer and his group. We cannot.

Dr. Waring: Dr. Irvine, would you conclude?

Dr. Irvine (Oxford, England): The question has been raised by one or two people whether the Mantoux test reaction is in any way qualitatively different or distinguishable following a natural infection compared with the reaction following BCG vaccination. It depends entirely on one's own experience, and I would say that particularly vole vaccinations which we have been following for three or four years tend to result in a rather flat, bluish Mantoux reaction which gives you great headaches in reading to decide whether it isn't a pure negative. That is about all I can say. In other words, there is a tendency for a peculiar type of Mantoux reaction to occur as the reaction is fading, but, if you were to give me a test and ask, is this a natural infection or is this after BCG, I should not be able to

tell you. In fact, I have tried it on myself, and made a complete fool of myself, so I back up Dr. Palmer completely—one cannot regularly distinguish between them.

Dr. Mitchell asked about the Heaf test at two days. I am afraid it is not possible to obtain a reliable reading in two days. It is a test which seems to come up slowly, and three days is the earliest that I dare to read it at all.

Finally, Dr. Dubos, it is a bold clinician who enters the bacteriologist's territory to fight him on his own ground. But I understand that Dr. Wells considers that, if you put vole bacilli into a vole, and, when the vole is dying, you reculture them from the vole and give the culture a different strain name, and do that after every ten generations of culture *in vitro*, you are not doing something which is called passage through an animal compared with putting these BCG into a guinea pig, and if possible, trying to fish them out again before they have died. Surely explicit passage through an animal cannot be used in the second instance. But here I merely pass along the information, being but a clinician.

Dr. Dubos: Animal passage is the perfect technique to change the characteristics of a culture.

Dr. Irvine: I am entirely defeated.

Dr. Waring: I think we should conclude this session.

## Session 3

Thursday Morning, November 10, 1955

ESMOND LONG, *Honorary Chairman*

GARDNER MIDDLEBROOK, *Moderator*

# IMMUNOPATHOLOGY OF TUBERCULOSIS[1]

SIDNEY RAFFEL[2]

At the time when the participants in this symposium were called upon for their first intimations of subject matter to be presented, I happened to be rereading, in Mark Twain's *Sketches New and Old*, an especially cogent one entitled "Some Learned Fables, for Good Old Boys and Girls."

In this are related the adventures befalling a scientific commision sent out by the creatures of the forest "to verify the truth of the matters already taught in their schools and colleges and also to make discoveries." After some weeks the expedition looked upon the unknown beyond its forest and soon came upon two seemingly endless parallel bars composed of a hard black substance. These, after suitable study, were finally declared by Professor Mudturtle, the most eminent geographer of his time, to be parallels of latitude. On the night of this discovery, a vast brilliant eye with a long tail attached shot by with great clattering and shrieking, and disappeared into the night. This caused great turmoil among the camp laborers, but not among the scientists; they remained calm, and after suitable deliberation pronounced this the Vernal Equinox. No sooner had this decision been rendered than the apparition came forth again, this time going in the opposite direction. Although this reversal was temporarily upsetting, it was given careful consideration by the best minds present, and eventually was recorded as the transit of Venus. When it was pointed out that Venus should traverse the sun's and not the earth's surface, Lord Daddy Longlegs, who had made the interpretation, pointed out that this was the crux of the discovery; that, although all who had lived before thought that a transit of Venus consisted of a flight across the sun's face, they believed it because of the limitations of their knowledge, while "to us has been granted the inestimable boon of proving that the transit occurs across the earth's face, for we have seen it."

Mark Twain's skepticism of science in 1875 was better founded than it would be today, and in any case I do not intend to suggest that his cynicism has bearing upon this Symposium, but I must confess to being struck by certain similarities between Twain's fable and my own contributions to today's proceedings.

What I have to say will deal with the tuberculous process at two points: first,

[1] The work forming the basis for this report has been supported by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association, made possible by a special bequest from the estate of Sophie Goman; and by the California Tuberculosis and Health Association.

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with initiation of the infection as an effect of the *virulence* of the tubercle bacillus; and second, with the relationship of the development of hypersensitivity and intensified antibody responses to the granulomatous process.

## VIRULENCE OF THE TUBERCLE BACILLUS

Without reviewing here the various facts and speculations which the literature of the past few years embodies with respect to factors relating to the virulence of this organism (1, 2), I should like to describe three lines of evidence which we have been following in our laboratory dealing with distinctions between virulent and avirulent strains of bacilli. We are not certain how far these observations may directly explain the ability of virulent organisms to propagate themselves in the host and the inability of avirulent strains to do so. Nevertheless, they reveal in sum a general capacity of the former to adapt themselves readily to circumstances with which avirulent variants are poorly equipped to cope.

The first observations have to do with the relative abilities of virulent and avirulent bacilli to multiply *in vitro* in the presence of small percentages of oxygen in the gaseous environment (3, 4). As shown in figure 1, a number of virulent strains of the *hominis* variety of this bacillus, for the most part recently isolated from sputum, were hindered in their multiplication in Dubos-Tween® medium in an open system through which an atmosphere containing 1 per cent oxygen

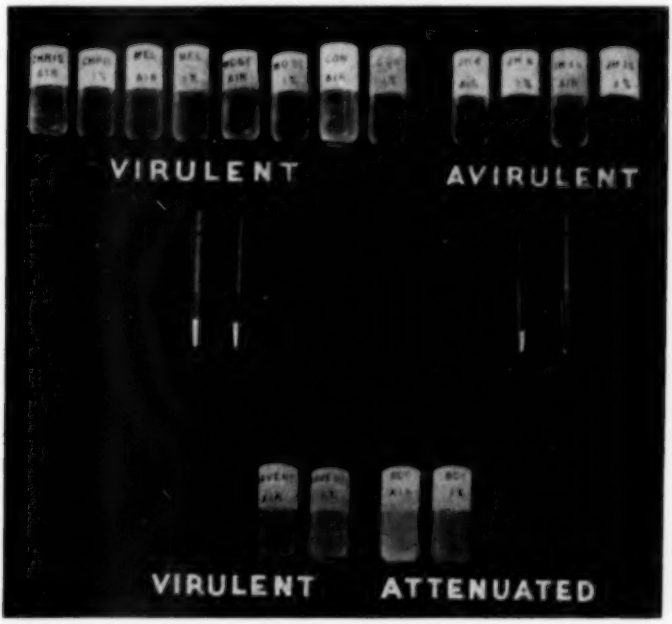


FIG. 1. Growth of virulent and avirulent tubercle bacilli in atmospheric and 1 per cent oxygen environments.

flowed continuously. The degree of this inhibition, however, was much less severe than that seen with avirulent strains under the same conditions. Interestingly, the attenuated strain BCG seems to be intermediate in this respect, with a closer relationship to the virulent than the avirulent strains. The point should be made that, although this figure deals with recently isolated human strains, the same effect was seen with laboratory strains, such as H37Rv, which had been maintained for long periods in culture medium.

In view of repeated suggestions by investigators in the past concerning the possible influence of oxygenation of the tissues upon the localization and growth of tubercle bacilli in animals and man (5–9), it seemed pertinent to inquire whether more direct evidence of a relationship of virulence to the capacity to utilize low concentrations of oxygen might be obtained in animal experiments. We have garnered some information suggesting that, to a limited degree, this may be so. If guinea pigs inoculated with avirulent organisms are subjected continuously to a gaseous environment in which the $pO_2$ is high, so that greater than ordinary quantities of oxygen are brought to the tissues (10, 11), there is some tendency for avirulent organisms to proliferate for a longer time and to a greater extent than is ordinarily the case.

A second study has had to do with the adaptability of various strains of the tubercle bacillus to new nutritional environments. We were led into this through an interest in the activities of macrophages upon tubercle bacilli. Suter (12) has reported that the macrophages of normal animals in tissue culture explants are able to suppress the propagation of avirulent but not of virulent tubercle bacilli. Mackaness (13) was unable to confirm this observation, or at least the interpretations deriving from it. He believes that the failure of growth of avirulent and even of attenuated bacilli is an expression of prolonged lag phases of growth of these organisms. Mackaness' thoughts in this matter were not clear to us. Prolonged lag phases as physiologic characteristics of organisms might be interpreted in one way, whereas a prolonged lag phase imposed upon an organism by the fact that it is inside a macrophage could coincide with Suter's interpretation that the normal macrophage interferes with bacillary growth.

We decided to examine this matter from the standpoint of possible differences in adaptability of virulent and avirulent strains of organisms to growth in several lifeless media, with the purpose of relating these observations to those made in experiments with macrophage cultures. The media employed for this study have been Dubos-Tween, the Hanks's solution-serum nutrient medium employed for tissue cultures, and a glycerol containing veal infusion broth. A number of virulent and avirulent strains which had for some time been adapted to growth in Dubos-Tween medium were transferred at five-day intervals, and growth curves were established. All strains were then transferred in measured quantities to all three media.

The result was quite striking. The curves of growth of virulent organisms in the two new media were somewhat more retarded than those seen in the medium to which they were accustomed, but the difference was much more pronounced in the case of the avirulent organisms, with which, in some instances, growth in the new milieus failed entirely. A sampling of this effect is shown in figure 2.

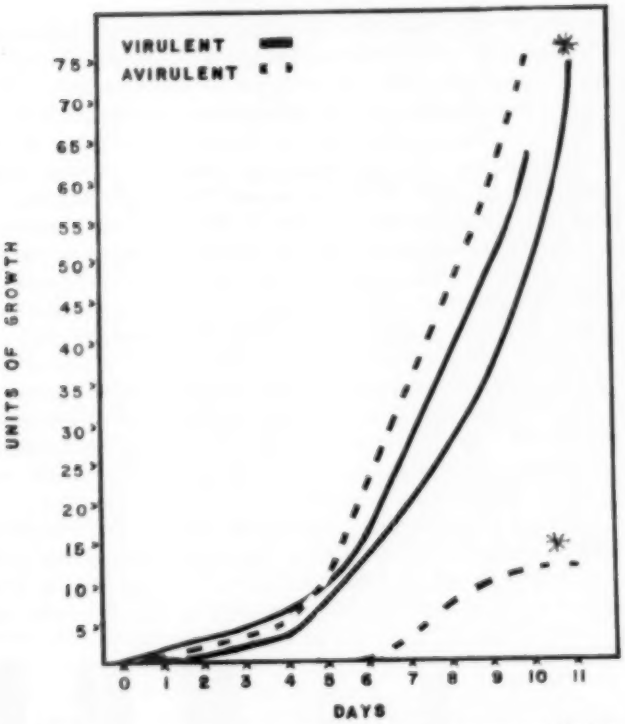


FIG. 2. Adaptation of virulent and avirulent bacilli to a new nutrient medium.
* Heterologous medium.

Through prolonged cultivation and frequent transfer in the new media, all strains were brought to the point of satisfactory growth under these conditions; on subsequent transfer to the heterologous media, in each case the same effect again appeared. These three media were chosen as random representatives of different nutrient environments and no special significance attaches to their particular qualities in this respect. It seems probable, however, that these results express a fundamental difference between virulent and avirulent organisms in the ability to propagate under strange nutritional circumstances. It is difficult to know whether one should think in this instance of differences in flexibility of enzymatic adaptations, of differences in stability of genetic constitution, or of some other mechanistic interpretation.

The third line of evidence relating to virulence is, in its details, unlike the preceding two. Nevertheless, it shares with them the basic feature of distinguishing differences in behavior of virulent and avirulent tubercle bacilli to a factor of the environment, in this case penicillin. Again, we were led to this study circuitously, through the puzzling failures of growth of avirulent, in contrast to virulent, organisms in samplings of fluid obtained from semipermeable capsules implanted in the peritoneal cavities of animals. Because of the manipulations

required in such samplings, penicillin was added to the plates of Dubos oleic-acid-albumin agar employed for quantitative colony estimations. In some instances 100, in other instances 200, units per milliliter of penicillin were used and, although virulent strains were found to grow in numbers consonant with our microscopic observations of the sampled materials, we often observed no growth whatever of avirulent strains, although these, too, appeared in large numbers in stained preparations. Because of the residence of the sampled organisms of both kinds in small capsules within the peritoneal cavity for variable periods of time up to three months, differences in cultural recovery of bacteria could well be related to the problem of recovery of bacilli from closed pulmonary cavities, with all of its implications of local inhibitory factors. Nevertheless, we believed it worth while to look into the question of a possible differential influence of the penicillin employed in the culture medium upon the two kinds of organisms.

As is well known, penicillin has no marked activity against virulent tubercle bacilli and, so far as I am aware, only virulent organisms have received attention in this respect in the past. There are quantitative differences in opinions on this point, some investigators (14–16) finding no significant effects with concentrations under about 100 units per milliliter; others (17) noting that smaller concentrations, between 20 and 80 units, may inhibit the growth of bacilli obtained directly from patients. Kirby and Dubos (18) found that in the presence of Tween 80 virulent bacilli may be susceptible to as little as one unit of penicillin per milliliter of medium, but that, in the absence of this detergent, 100 units

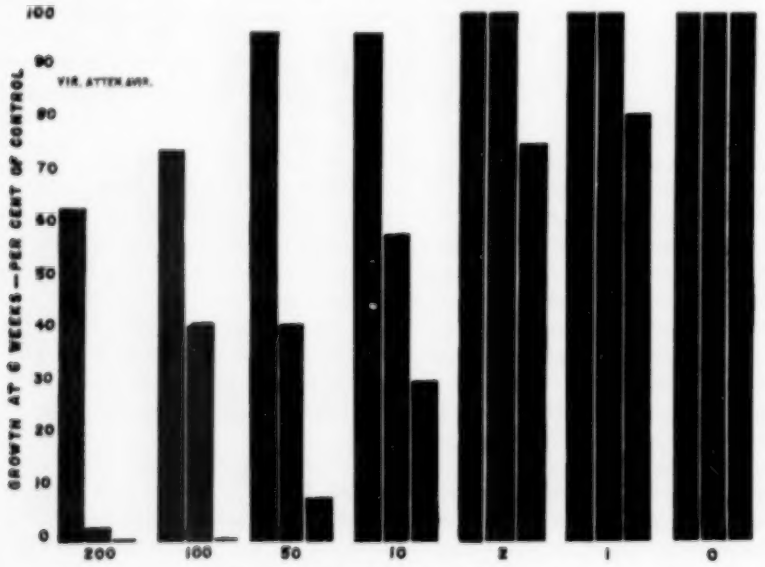


FIG. 3. Susceptibility of virulent, attenuated, and avirulent tubercle bacilli to penicillin.

were without significant antibacillary effect. The tenor of most observations permits the generalization that the susceptibility of the virulent tubercle bacillus to penicillin is such as to eliminate it from consideration as a therapeutic agent. The results, likewise, have favored the use of penicillin, at least in moderate concentrations, to suppress unwanted microbes in diagnostic or experimental procedures.

In a series of experiments carried out by Uyeda, Higashi, and myself, variable concentrations of penicillin ranging from one unit to 200 units per milliliter were incorporated in solid and liquid media, and these were inoculated with a number of virulent, avirulent, and attenuated strains of the bacillus. It soon became clear that avirulent bacilli are many times more susceptible to penicillin than are virulent organisms, as is indicated by figure 3. Virulent strains may be affected to a varying but limited degree by 100 units of penicillin per milliliter; in some instances there is only modest inhibition by 200 units. In contrast, the avirulent strains were generally strongly inhibited by 10 units of the agent, and in most cases they were substantially inhibited by penicillin concentrations of 2 units per ml.

* * *

The three kinds of experiments described permit very little in the way of reasonable correlative speculation about the intrinsic mechanism which endows the virulent organism with the power to make its way in the tissues, while its avirulent sibling falls by the wayside. But these observations may contribute to our understanding in this general sense: that in the case of this bacterial species the avirulent variants are those which are less able to propagate under a variety of environmental circumstances in which the virulent organism can continue to multiply quite effectively. The feature or features which determine these differences *in vitro* may well answer the question of pathogenicity *in vivo*.

## CELLULAR RESPONSES AND TUBERCULOUS ALLERGY, IMMUNITY, AND THE ADJUVANT EFFECT

Once infection has become established there follows within a short period of weeks the allergic state characteristic of tuberculosis (as well as of other infectious diseases) which is commonly referred to as delayed hypersensitivity. During this same early period immunity is acquired. Moreover, at the same time (19–22), and especially after deposition in the tissues of killed tubercle bacilli or appropriate components suspended in paraffin oil (23–25), an intensified humoral antibody response also develops to the antigens of the bacillus or to other antigens incorporated in the mixture. Certain cytologic changes likewise take place at this same time. They include the development of a granuloma and—especially under conditions of introduction into the tissues of bacillary components in oily emulsion (31)—of plasma cells.

The question is, how these three simultaneous immunologic developments—acquired resistance, allergy, and intensified antibody formation—may be related to the cellular changes and to each other. At the moment, our own information concerning these relationships is inadequate in certain areas. Nevertheless,

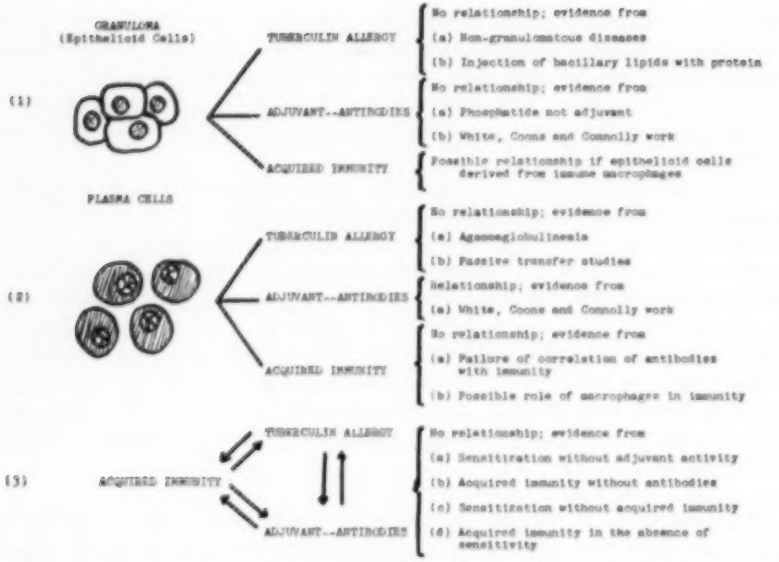


FIG. 4. Diagrammatic representation of cellular and immunologic responses to the tubercle bacillus.

through the use of collateral evidence supplied by other investigators and by other methods, I should like to discuss what appears to me to be the status of this complex question. A diagram (figure 4) will be used as an aid to the discussion.

*The granuloma:* Let us first consider the granuloma in relation to hypersensitivity. There are superficial reasons for thinking that the cells so characteristic of tuberculosis and a limited number of other infectious processes may also be involved in the production of the peculiar presumptive antibodies which establish this hypersensitive state. That epithelioid cells are most probably not the source of this immunologic response, however, is strongly indicated by the fact that hypersensitivities of the same kind develop during the course of many nongranulomatous infections. We decided, nevertheless, to make a direct study of this question with isolated lipoidal constituents of the tubercle bacillus along with tuberculoprotein as the sensitizing antigen.

The hypersensitivity "directing" substance of the tubercle bacillus is a lipoidal factor (26, 27) which exists in the bacterium as an ester of polysaccharide and mycolic acid. It is probable, however, that any ester of mycolic acid may possess the same activity (28, 29). This lipid is also very potent in provoking the epithelioid cell response (30, 31). Another lipoidal constituent of the organism which induces granuloma formation is found in the extracted phosphatides, as shown some time ago by Smithburn and Sabin (32). In past years we have had some difficulty in obtaining this material sufficiently free of the hypersensitivity-

inducing lipopolysaccharides to permit definitive work of the kind being described. Recently, however, Dr. Lederer was able to supply us with this portion of the bacterium entirely free of mycolic acid esters (33, 34).

Embach and I set up experiments in which groups of guinea pigs were inoculated in the foot pad with these two lipoidal substances, each along with protein in water-oil emulsion. At appropriate intervals tuberculin tests and bleedings were performed, and the animals were sacrificed to permit histologic examination of sites of injection as well as contiguous and distant lymph nodes and various organs. These examinations revealed that, although both of the lipid-protein combinations had induced local as well as some distant foci of granuloma (the lipopolysaccharide being superior to the phosphatide in this respect), only the lipopolysaccharide-protein mixture had simultaneously led to the development of tuberculous hypersensitivity.

In view of the facts that the tuberculin type of hypersensitivity can occur in the absence of granuloma and that granuloma can exist without the development of hypersensitivity, it seems reasonable to conclude that the epithelioid cell in itself is not, despite its rather special cytologic nature, the source of the equally special kind of hypersensitive state characteristic of tuberculosis.

We come next to the question of relationship of excessive humoral antibody formation to this same cellular response. Two lines of evidence indicate that such a relationship does not exist. First, in the experiment just described the lipopolysaccharide acts as an adjuvant to antibody formation, but the granuloma-inducing phosphatide does not. Second, it has recently been demonstrated by White, Coons, and Connolly (31), using the fluorescent marker technique, that in animals vaccinated with an adjuvant mixture containing lipopolysaccharide, antibody does not appear in the cells of the granuloma per se, but rather in plasmocytes which develop in large numbers, especially in lymph nodes distant from the site of injection. Here, too, we have converse lines of evidence to suggest a failure of relationship of granuloma to the immunologic response under consideration. Granuloma induction does not lead to intensified antibody production and, when the adjuvant effect occurs, the antibodies are not found in epithelioid cells.

Finally, there remains the question of the possible relationship of the granuloma to acquired immunity. The tentative answer here is not a straightforward one. The bacillary constituents with which we have induced granulomata do not provoke resistance. However, the epithelioid cell in itself represents a nonspecific response to any of a variety of stimuli. Thus, the mere fact of the presence of granuloma in the absence of immunity to tuberculosis by no means signifies that immunity cannot be intimately related to this cellular response in appropriate circumstances. For example, evidence has been presented by Lurie (37) and by Suter (12) that macrophages of animals immunized with bacillary vaccines possess an acquired ability to suppress the growth of tubercle bacilli. This evidence is not unanimous (13, 39*a*) but, if it proves to be true, then the cells comprising the granuloma in *immune* animals should be expected (because they derive from macrophages) to share their immune properties. In the instance of the granu-

loma induced by *nonimmunizing* constituents of the bacillus which we have employed, the cells comprising it do not have immune properties.

*The plasma cell:* So far as I am aware, no special study of plasmocytes in relation to the tubercle bacillus has as yet been made; the work of White and associates (31) mentioned before was carried out with isolated components of the organism. However, the possible bearing of these cells upon the various immunologic accompaniments of tuberculosis is suggested by certain indirect experimental and clinical observations.

In respect to tuberculous hypersensitivity, the report of White and co-workers (31) as well as earlier work (35, 36) make it seem very likely that the excessive antibody responses to antigens injected along with tubercle bacillary lipopolysaccharide in oil may well be explained by the intense plasmocytogenesis occasioned by this substance. The question which arises then is whether these cells may also explain the development of the hypersensitive state. Several kinds of information bear upon this point.

The special kind of hypersensitivity of which the tuberculin type is a classical example is generally considered not to be associated with circulating antibodies. However, Cole and Favour (39*b*) have recently reported experiments which suggest that tuberculous hypersensitivity may be transferable by an alpha-globulin fraction of the serum. If this work stands the test of time, the inference could be made that this humoral antibody concerned in tuberculous allergy is a product of the plasma cells which Coons and his colleagues (31) have observed following the inoculation of lipopolysaccharide and antigen in oil. On the other hand, the fact that the antibody described by Cole and Favour is unusual with respect to chemical composition might mean that it could be elaborated by cells other than those concerned in ordinary humoral antibody responses. At the moment, therefore, the interesting report of Cole and Favour cannot supply crucial evidence on the question at issue.

Investigations of another kind carried out in patients with agammaglobulinemia may be more pertinent. Good and Varco (40) have described extensive cytologic studies of a group of patients with this disease. These subjects were found to be devoid of plasma cells, even in lymph nodes draining sites of bacterial vaccination. It is well known from animal as well as human (40) studies that a considerable plasma cell response customarily occurs in the lymph nodes regional to a vaccinated area. The fact that agammaglobulinemic patients are devoid of plasma cells and are also unable to manufacture conventional humoral antibodies makes them most interesting subjects with respect to their capacity to develop the tuberculin type of hypersensitivity.

Good and Varco's data in general were concerned chiefly with another manifestation of such hypersensitivity, namely, to the streptococcus, and in this case the subjects revealed deficiencies of reactivity. However, one of their patients gave evidence of a positive tuberculin response. The reaction was atypical, manifesting itself as a protracted erythematous lesion without induration. These investigators decided that this was an insufficient basis for setting aside the conclusion that bacterial sensitivity, like humoral antibody fabrication, fails to

develop in the absence of plasma cells. There are, however, considerations which indicate that final judgment on this point may still be premature.

Recently I learned through personal communications of two definite instances of positive reactivity to tuberculin in patients of this kind. Doctors H. H. Zinneman and W. H. Hall of the Veterans Hospital in Minneapolis have observed a 35-year-old man lacking in gamma globulin and in humoral antibodies (isohemagglutinins). The patient's history included a laparotomy several years earlier, during which scattered intestinal serosal granulomata were found, as well as caseation and granuloma formation in a mesenteric node; acid-fast bacilli were not demonstrable in any of these locations. This patient responds with typical reactions to tests with tuberculin.

The second instance was that of a child studied by Dr. Herbert Porter on the Pediatric Service of Dr. Edith Lincoln at Bellevue Hospital. This child was not completely lacking in gamma globulin, but its concentration was less than 1 per cent of the normal value. Vaccination with BCG was followed after ten weeks by positive reactivity to Old Tuberculin in 1:10,000 dilution. During the time when this patient was under study, gamma globulin was injected as a prophylactic agent. In view of repeatedly observed failures of whole serum or gamma globulin to transfer tuberculin hypersensitivity, however, it seems highly improbable that the treatment could be related to the development of the reactivity noted.

These are as yet scanty data upon which to base skepticism concerning the role of the plasma cells in the genesis of tuberculous hypersensitivity. But other supportive evidence comes from cellular transfer studies in animals. Chase (41) has shown that animals vaccinated with picryl chloride mixed with dead mycobacteria develop both antibodies and delayed hypersensitivity to the chemical agent. Moreover, the lymphocytes of such animals transfer the antibody-producing capacity as well as the hypersensitivity to normal recipients. On the other hand, donors vaccinated with a picryl chloride-protein conjugate develop humoral antibodies without delayed hypersensitivity and their lymphocytes transfer only the antibody-producing ability to recipients.

It seems strange that the immunologic responses to the same antigenic chemical substance should differ, depending upon the vehicle in which the antigen is presented to the tissues, if the same cell type responds in both circumstances. Yet, it is true that, in both cases, the same cells, the lymphocytes, appear to transfer the appropriate immunologic reactivities to normal recipients. An explanation may be that, whereas the transferring cell may endow the recipient with a *potential* ability in the case of antibody production, i.e., with an initiator of a response in which the recipient takes an active part (42), it may, in the instance of delayed reactivity, be transmitting merely an accomplished state. This would amount simply to a passive "implantation" of already sensitized cells in the body of the recipient, and it is these cells which give rise to the reaction when the recipient is tested with antigen. This possibility is suggested by the fact that leukocytes other than lymphocytes can also transfer delayed hypersensitivity (43), but not the antibody-forming ability.

The fact that such antibody suppressing agents as irradiation (44) and nitrogen mustard (45) will also suppress or postpone the ability of the animal to respond to tubercle bacilli with tuberculin hypersensitivity suggests superficially a relationship between the genesis of humoral antibodies and bacterial hypersensitivity. Information of this kind is difficult to evaluate at this time because the damage occasioned by both agents is not localized to single cell types. Hence, it is impossible to know whether one or multiple aspects of immunologic responses may be affected.

The time is not ripe for conclusions in the matter of the relationship of plasma cells to the development of bacterial allergy. It is my own tentative opinion that the cells of most importance in humoral antibody formation may not be those which are most concerned in the genesis of the tuberculin type of hypersensitivity. Opinions in this field, however, are not especially pertinent, since the degree of interest in this and related questions and the ever increasing delicacy of methods brought to their study promise a factual resolution of the problem in the near future.

The possible role of the plasma cell in acquired resistance to tuberculosis hingeson the question of whether humoral antibodies are concerned in the process. If so, then it would seem most likely that these cells which have been implicated in the antibody-producing process by so many studies would be concerned here also. But there is considerable doubt that humoral antibodies are concerned in acquired immunity to this disease (2), as suggested by serologic, passive transfer and other studies (46). The evidence for this negative viewpoint is perforce incomplete. However, if the evidence is considered along with earlier cited evidence for the probable role of macrophages in the immune state, it gains persuasiveness. The issue is certainly not a closed one; tentatively, however, it appears unlikely that the antibody-producing plasma cells are concerned in acquired resistance to this disease.

To sum up the relationship of plasma cells to the three immunologic responses considered: these would seem undoubtedly to be the main or only source of circulating antibodies against components of the bacillus, as judged by the anti-protein response (31). Likewise, there is serious doubt that they provide the immunologic basis for tuberculin hypersensitivity. Finally, there is even more serious question that these three immunologic responses provide the mechanism of acquired immunity to the tubercle bacillus.

* * *

Having questioned the relationship of tuberculous hypersensitivity, of humoral antibody formation, and of acquired immunity to the cells of the granuloma and to plasmocytes, we turn now to the possible relationship of these immunologic responses to one another. Is the antibody adjuvant effect, provided by mycobacteria or their constituents under appropriate procedural conditions, related to hypersensitivity induction in the sense that the latter may be in some way predicated upon the former? As is evident from the preceding discussion, I believe that the answer to this is a negative one, since the two immunologic phenomena probably do not have a common cellular origin, although they are

common effects of the same cause, tubercle bacillary constituents. The combination of lipopolysaccharide and protein of this bacterium can provoke greater than ordinary numbers of plasma cells to synthesize large quantities of humoral antibody, and it can apparently implement some other mechanism at the same time to elaborate the hypersensitive state.

Subsidiary evidence for this viewpoint, in addition to that already discussed, includes the fact that tuberculin hypersensitivity may be induced in the absence of any detectable humoral antibody response whatever. Crowle and I have observed this phenomenon following the inoculation of killed tubercle bacilli obtained from sputum by methods which cause partial deterioration of the proteins of the organism. Moreover, we have noted many times in the past (26) that the lipopolysaccharide-protein mixture, when suspended in saline rather than in oil, provokes tuberculin sensitivity without acting as a stimulant to excessive humoral antibody synthesis. These observations strongly suggest that tuberculous allergy is not simply a facet or consequence of exuberant antibody fabrication.

The adjuvant effect appears also to be unrelated to the acquisition of immunity, for the evidence so far presented does not favor the viewpoint that tuberculous immunity depends upon humoral antibodies.

Finally, the relationship of hypersensitivity to immunity in this disease has been discussed on many occasions. Induction of the two states depends upon changes which are not yet clearly understood but, as discussed before, are probably not common to these two events. If this is the case, then the hypersensitive state should not be expected to constitute immunity, an opinion which is rather generally held at the present time for a variety of reasons. Prominent among these are the facts that immunity persists in vaccinated animals after sensitivity has waned (48); that desensitization of vaccinated animals leaves immunity intact (49); and that hypersensitivity may be induced in the absence of immunity (26*b*). However, one reservation should be made. If in the hypersensitive animal re-exposure to bacilli means the more rapid formation of a granulomatous response and if the cells participating in this response are themselves immune, then in an indirect fashion this facet of hypersensitivity may make immunity quantitatively more effective than it would otherwise be.

## GENERAL SUMMARY

The substances of the tubercle bacillus which induce tuberculin allergy give rise at the same time, under appropriate circumstances, to large quantities of humoral antibodies. They provoke as well two outstanding cytologic responses, one macrophagic; the other plasmocytic. The large numbers of plasma cells are probably responsible for the excessive humoral antibody manufacture (the adjuvant effect), but it seems doubtful that either of these cell types takes part in the development of the hypersensitive state. Other constituents of the bacillus, not yet clearly defined, induce acquired immunity, which in turn appears to be independent of allergy as well as antibody synthesis, both in cellular origin and in immunologic nature.

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# PATHOGENICITY OF ISONIAZID-RESISTANT TUBERCLE BACILLI AND PROPHYLAXIS OF TUBERCULOSIS IN CHILDREN

NOËL RIST[1]

Soon after the introduction of isoniazid, the phthisiologist met with two disappointments: the frequent appearance of isoniazid resistance and the difficulty of delaying it for a long time with the usual combination therapy.

It was, therefore, with an evident feeling of relief that the physicians heard that most isoniazid-resistant bacilli showed a very reduced pathogenicity for the guinea pig. We should like to examine today whether this feeling of relief is already fully justified.

Speaking in a symposium on tuberculosis in childhood, we shall consider the pathogenic potential of the isoniazid-resistant, attenuated bacilli for children living with patients who eliminate these bacilli, and intentionally disregard the problem of pathogenicity of such strains for the treated adult patients themselves.

Although pathogenicity for guinea pigs does not necessarily mean pathogenicity for man, first we have to observe the behavior of the attenuated strains in the guinea pig. And, as we are dealing with an essentially practical problem, we have chosen to study not only pure strains, artificially selected by subculture on isoniazid-containing media, but the total bacillary populations as they are actually excreted by patients.

The technique is the following (figure 1): With the same sputum we make:

*An ordinary culture*, from which we can later infect the guinea pig with a known inoculum; *a direct inoculation to the guinea pig;* and *a direct resistance titration*.

The direct resistance titration is done with a diluted inoculum in order to get numerable colonies on the control culture, that is, from 50 to 400 and to know the percentage of bacilli resistant to each isoniazid concentration. We say that a given population is "totally resistant" to $n\gamma$ of isoniazid per ml. only if the tube containing $n\gamma$ per ml. shows as many colonies as the control. However, the irregularities inherent in any method involving plate counts compelled us to consider as "containing as many colonies as the control" any culture containing 50 to 100 per cent of the number of colonies of the control.

We shall consider today only those populations which seem to be totally resistant to isoniazid concentrations of 0.1 $\gamma$ of isoniazid per ml.

With usual titration techniques which involve a heavy inoculum and a confluent growth on the control, many mixed populations of tubercle bacilli seem to be "totally resistant" to some concentration of isoniazid, even when they contain only 10 per cent of resistant bacilli. The diluted inoculum technique provides evidence that such mixed populations contain many drug-susceptible bacilli. These obviously mixed populations do not enter the scope of our study. They are usually normally virulent.

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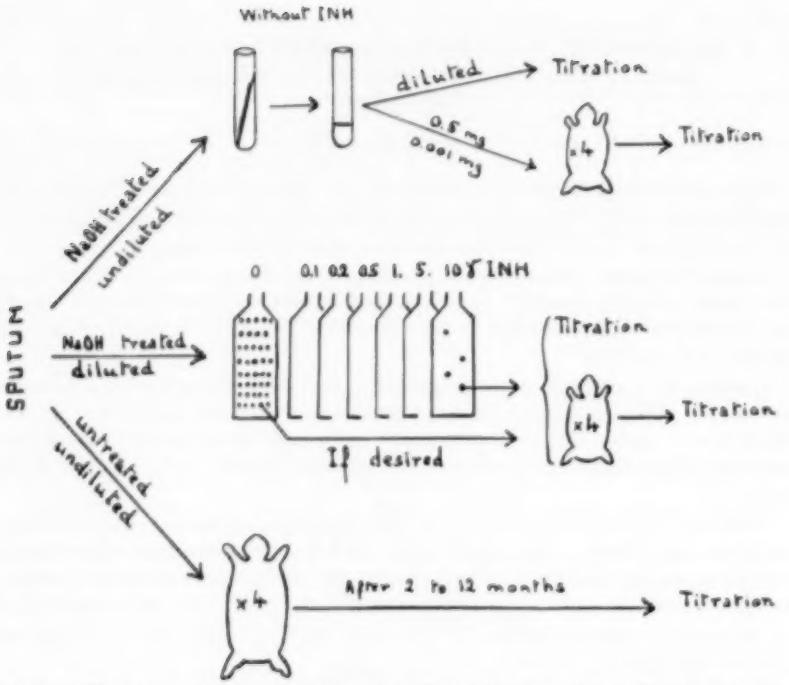


FIG. 1. Technique used to analyze the total population of tubercle bacilli in a sputum specimen.

As shown by figure 1, each time a group of 4 guinea pigs is inoculated, an aliquot part of the inoculated culture or sputum is diluted and titrated for isoniazid resistance. In addition, each time a guinea pig develops visceral lesions, suggesting the presence of virulent bacilli, the lesions are directly titrated after adequate dilution. It is this last titration that allows us to label each population and to describe three types of populations as follows (table 1).

*Type I populations:* Some populations of tubercle bacilli, although they seem to be totally resistant by the *in vitro* tests (totally resistant and catalase negative) actually are virulent; that is, they are able to cause a progressive lethal tuberculosis. But this is due to the presence of some drug-susceptible bacilli, unnoticed in the first *in vitro* titration.

Three to six months after the inoculation the resistant and attenuated bacilli are dead; the susceptible ones have multiplied. The titration of the lesions produced by such populations shows these susceptible bacilli almost exclusively (2*b*). Examples of this phenomenon will be found in a previous publication (3).

*Type II populations:* Some populations of tubercle bacilli are at the same time purely isoniazid resistant and virulent for the guinea pig. The titration of the lesions reveals only resistant bacilli.

*Type III populations:* The great majority of the populations of tubercle bacilli

TABLE 1

TYPES OF BACILLARY POPULATIONS ENCOUNTERED IN SPUTUM WHICH SEEMS, BY IN VITRO TESTS, TO CONTAIN EXCLUSIVELY ISONIAZID-RESISTANT BACILLI

| Type | Apparently Totally Resistant to Isoniazid | Number of Populations |
|---|---|---|
| | *γ per ml.* | |
| I. Virulent because of unnoticed sensitive bacilli: 7 populations | 20.0 | 1 |
| | 10.0 | 3 |
| | 1.0 | 3 |
| II. Virulent and resistant: 5 populations | 1.0 | 3 |
| | 0.5 | 1 |
| | 0.1 | 1 |
| III. Attenuated: 34 populations | > 10.0 | 12 |
| | 1.0 | 6 |
| | 0.5 | 9 |
| | 0.2 | 5 |
| | 0.1 | 2 |
| Total | | 46 |

recovered from the sputum of isoniazid-treated patients, and shown to be totally resistant in the first *in vitro* titration, are definitely attenuated. They provoke a generalized tuberculosis, but the visceral lesions (even those which are macroscopic) regress, leaving only local adenopathies. The titration reveals only isoniazid-resistant bacilli.

At first sight, this distribution of our forty-six populations was rather reassuring. It showed that the attenuation of virulence was not limited to selected, isolated colonies, but concerned also many of the total populations actually present in the sputum. It showed also that a resistance to 0.1 γ is often already sufficient to be accompanied by an attenuation of virulence.

But this outline, elaborated about one year ago (3, 4), was based on guinea pigs which were very seldom observed for more than six months. Since then, we have had the opportunity to observe new facts which made us more reluctant to assume the harmlessness of isoniazid-resistant populations of tubercle bacilli.

Accordingly, we shall consider successively each of the three schematic types of populations described in table 1.

*Type I populations*: *Predominantly drug-resistant but including some drug-susceptible numbers:* The seven *Type I* populations already showed us that the *in vitro* test does not allow us to assert that an apparently totally resistant population will not sometimes contain some susceptible and virulent bacilli. We believed, however, that these virulent bacilli should manifest their presence in all inoculated guinea pigs, and within six months after the inoculation.

We now know that they may appear later. The most typical example is the following:

Among 4 guinea pigs inoculated with 0.5 mg. of the same population of tubercle bacilli,[2] 2 were sacrificed after five months, without any visceral lesions, and the population was

[2] In fact, a population issued from a single colony picked out of a medium without isoniazid, apparently totally resistant to 10 γ of isoniazid and catalase negative.

labeled "resistant attentuated." After eight months, the third guinea pig died, with many visceral lesions full of susceptible bacilli. The fourth was sacrificed at the same date, without any lesions except a caseous inguinal lymph node, in which we had the surprise of finding only susceptible bacilli which proved normally virulent when reinoculated.

One may suppose that in this fourth guinea pig the few virulent bacilli present in the inoculum were inhibited by the immunity conferred by the bulk of the nonvirulent population.[3]

One might also suspect a reverse mutation. We know of no facts in our material in favor of that explanation.

Whatever the explanation, the fact is that, after a longer observation of the guinea pigs of our thirty-four *Type III* populations, originally assumed to be totally attenuated after *in vitro* titration and a three- to six-month observation period of the guinea pig, at least 6[4] revealed their potentiality of showing virulent bacilli after six to eight months' residence in a guinea pig. Why could this phenomenon not occur in a child?

*Type II populations: Both drug resistant and virulent:* The *Type II* populations, simultaneously resistant and virulent, are often considered to be exceptional because we pay attention only to highly resistant strains. As a matter of fact, highly resistant virulent strains are scarce, and we had not the good fortune to meet any one of them. Nevertheless, we believe that low-resistant strains may have very great clinical implications. Indeed, we have seen that a strain which is totally resistant to 0.1 $\gamma$ of isoniazid only (and contains some bacilli resistant to 0.2 $\gamma$ and to 0.5 $\gamma$) is, in the mouse, almost totally resistant to treatment with 25 mg. of isoniazid per kg., which merely delays death for some days. In contrast, 2.5 mg. of isoniazid per kg. totally inhibits the development of lesions when a drug-susceptible strain such as H37Rv is inoculated.

It is useful to note, moreover, that virulent resistant strains are virulent not only when they are very heavily inoculated. The following illustrations will show the lesions of guinea pigs inoculated with $10^{-3}$ mg. (figure 2) and $10^{-5}$ mg. of bacilli, that is, with about 1,000 bacilli (figure 3).

With regard to these weakly resistant strains, we quite agree with Middlebrook and Dressler when they say that, once they have made their appearance, a higher dosage of isoniazid does not eliminate them from the patient's sputum (5).

The only interest in continuing the treatment, in cases when the bulk of the population of tubercle bacilli has become resistant, could be the continuous elimination of the susceptible bacilli. But the continuation of the treatment may be harmful if one takes into account the enhancing of tuberculous evolution by isoniazid described in mice by Barnett (1) and by Grumbach (6), and their respective associates, and in man by Ogilvie (7).

[3] This fact teaches us that the absence of visceral lesions is not an evidence of the absence of virulent bacilli. Contrary to our usual technique, we should, therefore, re-titrate the bacilli whenever we find them and not exclusively in the visceral lesions.

[4] We write "at least" because in many cases when the guinea pig seemed to be free from visceral lesions, we had not the curiosity to re-titrate the bacilli found in the lymph nodes.

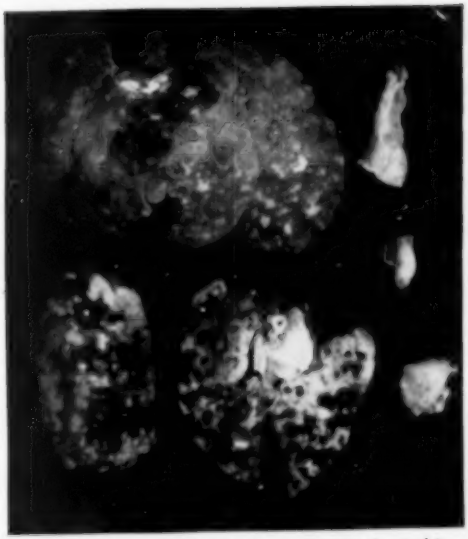

FIG. 2. Strain Ti. Totally resistant to isoniazid concentrations of 1 $\gamma$ per ml.; susceptible to 10 $\gamma$. Guinea pig which was inoculated with $10^{-2}$ mg. (about 100,000 bacilli) died four months after inoculation.

FIG. 3. Strain De. Totally resistant to isoniazid concentrations of 1 $\gamma$ per ml.; susceptible to 2 $\gamma$. Guinea pig was inoculated with $10^{-3}$ mg. (about 1,000 bacilli), and was sacrificed five and one-half months later. Other guinea pigs of the same series died about the same time.

FIG. 4. (Upper). Strain Bo. Totally resistant to isoniazid concentrations of 10 γ per ml. Thirty-three days after inoculation, lung was scattered with many epithelioid nodes.

FIG. 5. (Center). Guinea pig of the same series as figure 1. Seventy days after inoculation, the lungs healed. The tracheobronchial nodes were still necrotic.

FIG. 6. (Bottom). Strain Ni. Totally resistant to isoniazid concentrations of 10 γ per ml. Guinea pig was sacrificed 60 days after inoculation. A necrotic node was present in the lung. Other guinea pigs of this series were found normal six and eight months after inoculation.

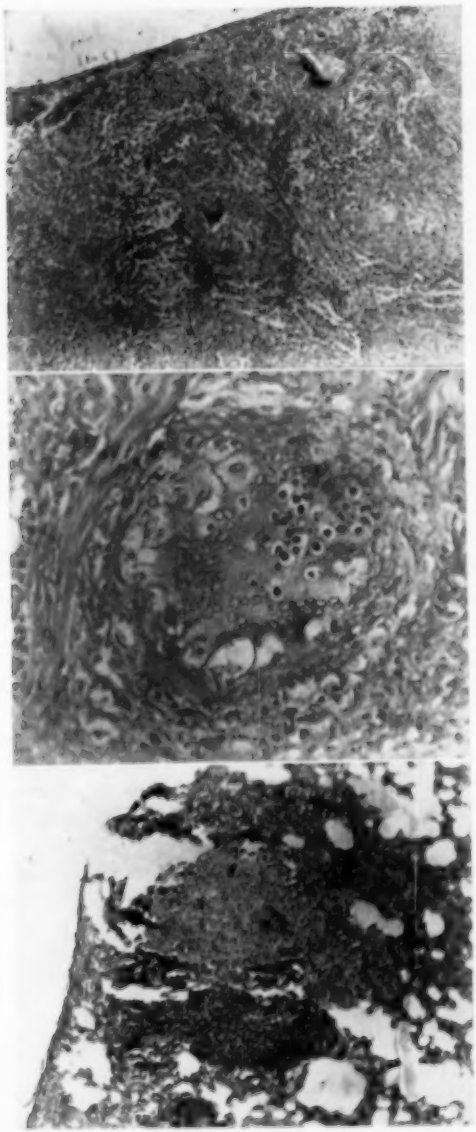

FIG. 7. (Upper). Strain Gui. Totally resistant to isoniazid concentrations of 10 γ per ml. Guinea pig was sacrificed 165 days after inoculation. The spleen was entirely filled with epithelioid nodules—big giant cells.

FIG. 8. (Center). Same spleen as in figure 7, showing vacuolated giant cell, similar to findings in human resected lungs, after isoniazid therapy.

FIG. 9. (Bottom). Strain Ma. Totally resistant to isoniazid concentrations of 20 γ per ml. Guinea pig was sacrificed 185 days after inoculation. Fibrotic nodule with giant cell was found, as well as a lymphocytic nodule.

*Type III populations: Composed only of drug-resistant and attenuated bacilli:* The *Type III* populations[5] of tubercle bacilli contain only isoniazid-resistant and attenuated bacilli. If we try to determine the *degree* of the attenuation of these tubercle bacilli, we are at once struck by its extreme diversity. Moreover, the diversity of this attenuation is independent of their degree of isoniazid resistance.

Two years ago (8) we had already pointed out that these attenuated strains could not be classified in the group of the true nonvirulent bacilli, such as BCG and H37Ra.

In the mouse, the difference is striking. All of our isoniazid-resistant attenuated strains kill mice under circumstances in which BCG and H37Ra are never fatal (3, 6). In the guinea pig, the difference is likewise evident. The lesions due to the attenuated strains are regressive, as are those of BCG; but they are initially much more extensive and much more lasting than the latter.

The best infecting dose to show these differential responses, in our experience, is about 0.5 mg. of bacilli moist weight (approximately 2.5 by $10^7$ cultivatable bacillary units), by the subcutaneous route. With that dose, the para-aortic lymph nodes are never caseous after inoculation of BCG or H37Ra; yet they are almost always either caseous or purulent six weeks after inoculation of an isoniazid-resistant attenuated strain.

The visceral lesions, never visible to the naked eye with BCG or H37Ra, are often spectacular with the so-called attenuated strains, and it may be difficult to decide, about the thirtieth to forty-fifth day, whether these lesions will regress or extend.

The severity of the lesions, as well as the speed of regression, varies greatly according to the strain, but independently of the degree of drug resistance. Illustrations are presented in figures 4–11 of some examples of lesions due to strains totally resistant to 10 $\gamma$ of isoniazid, catalase negative, and inoculated subcutaneously in doses of 0.1 to 0.5 mg. (moist weight).

The lesions are sometimes of the pure epithelioid type, as may be seen in figure 4. In this instance, although the lungs were scattered with miliary tubercles thirty-three days after inoculation, the lungs were normal at seventy days, the tracheobronchial nodes still showing important necrosis (figure 5).

Other lesions are conspicuously necrotic sixty days after inoculation, and one could doubt the possibility of regression (figure 6). In the particular series illustrated in figure 6, the 3 other guinea pigs had normal lungs six months after inoculation.

The healing proceeds usually by complete resolution in six to eight months, but there are many exceptions. In figure 7 may be seen the reproduction of a spleen which is still entirely replaced by epithelioid tissue, five and a half months after inoculation. Among the numerous big giant cells, one is reproduced (figure 8) which is of a quite typical shape and shows many vacuoles which seem to

[5] All that we say about *Type II* and *Type III* is equally true for isolated strains which have been selected on isoniazid-containing media and for populations which come directly from a patient's sputum and are free from drug-susceptible bacilli.

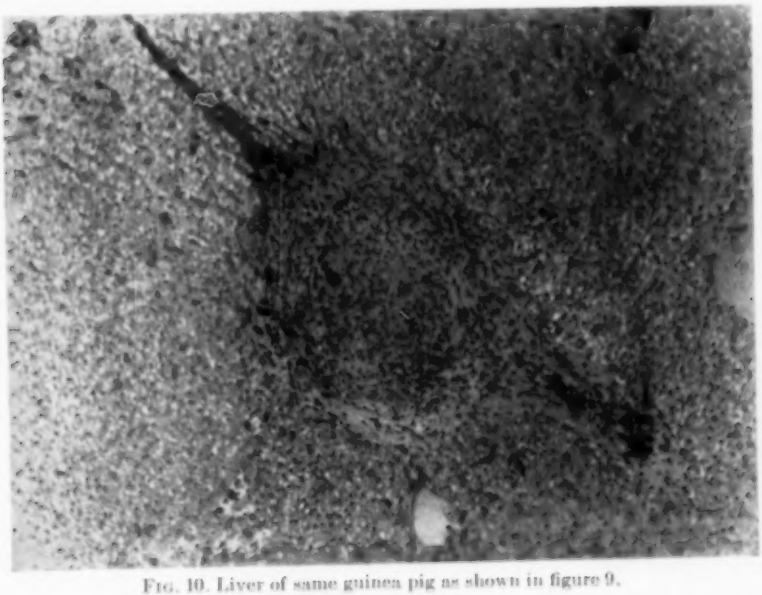

FIG. 10. Liver of same guinea pig as shown in figure 9.

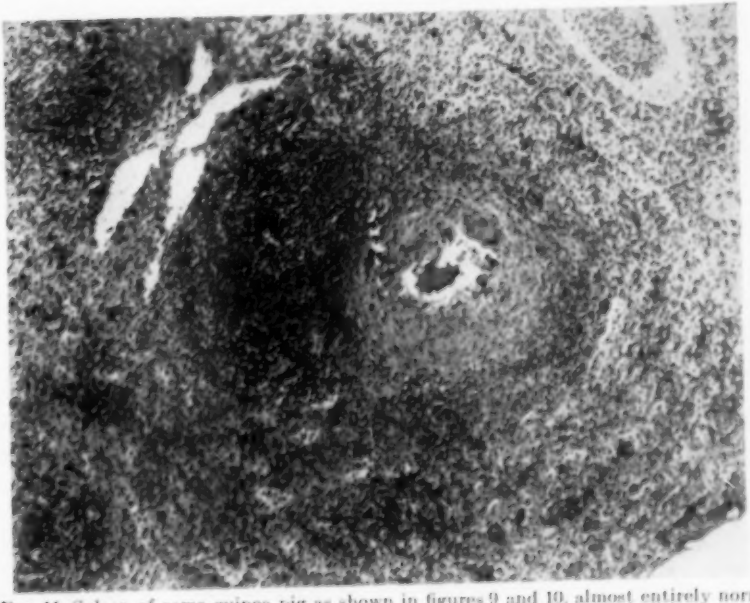

FIG. 11. Spleen of same guinea pig as shown in figures 9 and 10, almost entirely normal except some fibrotic nodules whose centers contained giant cells and early calcification.

contain debris of polymorphonuclear cells. This type of giant cell resembles some of the typical giant cells with vacuoles described by Canetti (9) in resected human lungs following isoniazid therapy.

Necrotic foci may also heal by formation of fibrous scars, which are still very conspicuous six months after the inoculation (figures 9, 10, 11).

Of greater importance, however, is the fact that, since we initiated the practice of inoculating a greater number of guinea pigs and observing them for more than six months, we have learned that the law of regression itself has exceptions of two kinds (10). First, we have seen that before the regression takes place, in about the second or third month, there is a critical phase during which some guinea pigs may die of acute miliary tuberculosis, while all of the other animals which have received the same dose of the same strain will be completely healed four to eight months later.

Second, the lesions regress slowly in all organs, but in one of them one finds a solitary focus of what can be called "adult type" tuberculosis. For instance, seven to ten months after the inoculation of a catalase-negative strain, we have found a voluminous liver abscess (while the lung was only scattered with lymphoid discs); a solitary hypertrophic tuberculosis of the spleen; and, on three occasions, *solitary pulmonary cavities, analogous to human chronic pulmonary tuberculosis.*

The three instances of chronic cavities are presented in detail:

*First guinea pig:* Six months after the inoculation of $10^{-2}$ mg. of bacilli subcutaneously, two pulmonary cavities were observed (figure 12). The cavities were swarming with bacilli which, like the inoculated strain, were resistant to isoniazid concentrations of 1 $\gamma$ per ml. and were catalase negative. In the walls of the cavities there was some calcification. The tracheobronchial nodes were normal, except for some very small areas of calcification. The bronchi were filled with pus which was swarming with tubercle bacilli. The findings were closely similar to those in human chronic tuberculosis. The liver was normal and the spleen contained numerous big calcified foci.

*Second guinea pig:* Seven and one-half months after inoculation of 0.5 mg. of tubercle bacilli, two pulmonary cavities were noted in this animal. The first cavity was opened in the pleural space by the dissection (figure 13); the second was whole. The contents of the cavities were swarming with tubercle bacilli, which were totally resistant to isoniazid concentrations of 10 $\gamma$ per ml. and were catalase negative. The tracheobronchial lymph nodes were normal and there were some minute fibrotic follicles in the liver and spleen.

*Third guinea pig:* Seven months after inoculation of 0.5 mg. of bacilli, this animal showed one very small cavity which was perhaps infiltrated with calcium. Another cavity was lacerated during separation of the pleural space. On the edge of the lung there were several tuberculomata, and in each of them several tubercle bacilli, which on culture were totally resistant to isoniazid concentrations of 10 $\gamma$ per ml. and were catalase negative. The tracheobronchial nodes were almost normal, with a few minute epithelioid tubercles. The liver and spleen showed healed lesions.

These open, pulmonary cavities are of special interest. It is generally assumed that in human cavities the isoniazid-resistant, catalase-negative tubercle bacilli multiply as freely as normal virulent bacilli and, as stated by Oestreicher and his associates (11), "their poisonous characteristics have not been found to differ

FIG. 12. Strain Go. Totally resistant to isoniazid concentrations of 1 $\gamma$ per ml.; susceptible to 2 $\gamma$ per ml. (catalase negative). Guinea pig was sacrificed six months after inoculation of $10^{-3}$ mg. of bacilli. *See* text under "first guinea pig."

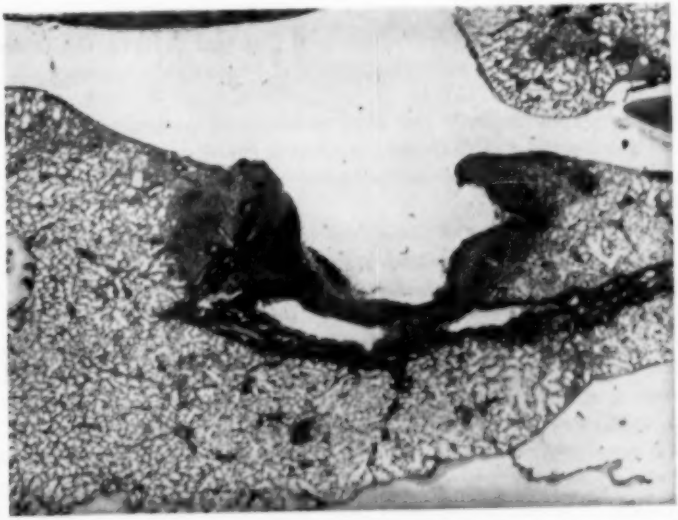

FIG. 13. Strain Ma. Totally resistant to isoniazid concentrations of 10 $\gamma$ per ml. (catalase negative). Guinea pig was sacrificed seven months after inoculation of 0.5 mg. of bacilli. *See* text under "second guinea pig."

from those of their fully pathogenic, catalase-positive parents." On the other hand, it is evident that, in the noncavitary lesions of the guinea pig, the isoniazid-resistant, catalase-negative bacilli are readily killed as soon as the guinea pig becomes allergic.

We have often tried to conceive a way to create cavities in the guinea pig to see whether in that situation the isoniazid-resistant bacilli would multiply as well as in the human cavities. Nature and chance have given us such cavities and the hypothesis has been confirmed that, provided they are in an open cavity, the attenuated catalase-negative bacilli multiply as well in guinea pigs as in human beings.

In mice, the situation seems to be different, since in that species the attenuated bacilli multiply and kill the animal, even in the absence of cavity. As to the fate of these bacilli in man, when there is no cavity, too little is known as yet to speak of this very important problem.

What is the cause of the formation of cavities in the 3 guinea pigs we reported? Is it a sudden rise of tuberculin hypersensitivity? Or an acute local nonspecific pulmonary infection? Either accident could happen in a child as well as in the guinea pig.

* * *

To summarize these facts, I should like to stress the extraordinary diversity of the isoniazid-resistant mutants *in vivo*, as has already been shown *in vitro*. Many experiments *in vitro* have already shown this diversity. The viability, the growth requirements (12, 13), and even the cross-resistance characteristics vary with the strains. For instance, the isoniazid-resistant mutants are usually, but not invariably, resistant to cyanacetic acid hydrazid (14); on the other hand, the same mutants are usually—but again not invariably—susceptible to isonicotinic acid thioamide (15).

*In vivo*, we have observed the same diversity in pathogenicity. And, if we combine the diversity of pathogenicities with the diversity of individual susceptibilities of hosts, we may easily expect an extreme diversity of pathologic responses.

Concerning the diversity of individual susceptibilities, I must confess that in our experiments this diversity may have been exaggerated by the fact that our guinea pigs do not come from a pure inbred strain. But, for that matter, neither do our children!

We have tried to express graphically the pathogenic situation of isoniazid-resistant, attenuated strains, which is intermediate between BCG and the normal virulent strains (figure 14). The various pathogenicities of the isoniazid-resistant strains cover the whole range between these two extremes.

In the most frequent evolution of infection with isoniazid-resistant mutant strains the regression is complete, but it may proceed either quickly (broken line on figure 14) or slowly (plain line). However, some guinea pigs fail to manifest the regression at all, and die in the third month of acute miliary tuberculosis. Others fail to carry the regression to its completion. The lesions proceed, as described, to chronic, cavitary pulmonary tuberculosis.

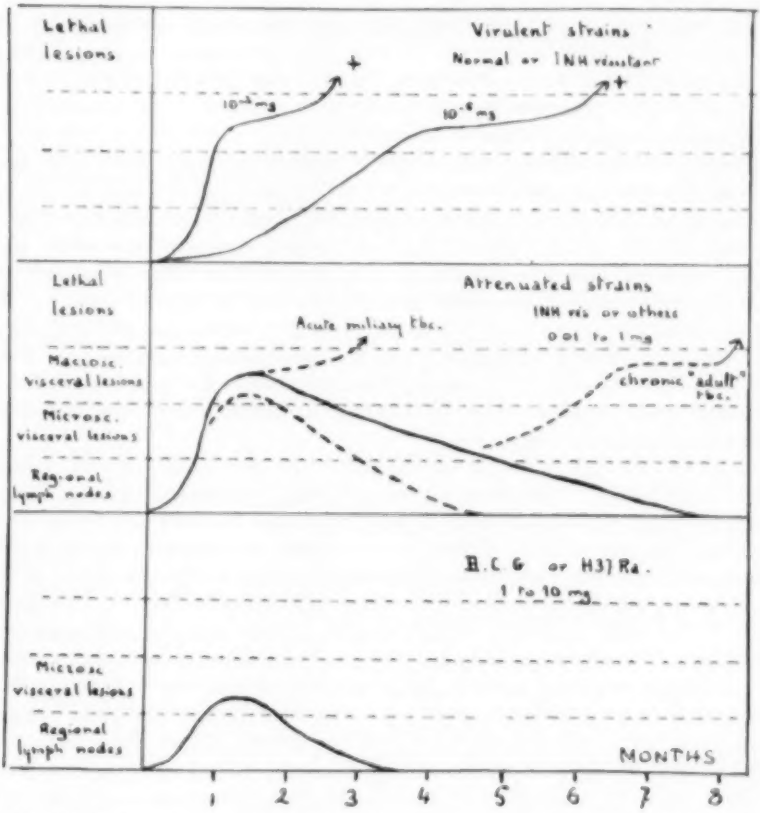


FIG. 14. Pathologic responses of guinea pigs to normal and to attentuated strains.

In short, guinea pigs manifest the same range of responses to these attenuated strains of tubercle bacilli as human beings do to fully virulent tubercle bacilli.

If we try to extrapolate to human beings, it is evident that one cannot reason about the attenuated strains as if they were like BCG. And the diversity of their pathogenicities forbids us to draw a *standard picture* of all of the strains which are labeled "isoniazid resistant," or to venture any general prediction of their behavior in man.

We must, therefore, turn to the clinician. The observations we have just presented about strains isolated from *treated patients* can only help us to know the great difficulties of a thorough analysis of a bacillary population. They can also serve to give a scheme of analysis to those who wish now to proceed to the next step, that is, the obstinate search for contact cases, possibly contaminated by isoniazid-resistant populations, and the analysis of strains eliminated by previously *untreated patients*.

The result may prove negative for some years hence; but the fact that it has been practically negative until now is still quite inconclusive if we recall what happened with streptomycin resistance. On Professor Debré's meningitis service in Paris (16), the first cases due to streptomycin-resistant bacilli appeared only three years after the introduction of streptomycin.

On the other hand, some thirty cases of lung tuberculosis which have been published as due to isoniazid-resistant strains are not conclusive either.

The most satisfactory case was recently described by Dr. Meissner (2*a*) in Borstel: A baby girl of sixteen months suffered a "caseous pneumonia" after contact with her tuberculous father who had been treated with isoniazid. In the father's sputum and in the infant's gastric washings, an identical strain of tubercle bacilli was found in that it was resistant to isoniazid concentrations of 50 $\gamma$ per ml., was catalase negative, and was attenuated for guinea pigs. However, the infecting contact apparently took place while the infant was ill with pertussis, which may have played a decisive role. Hence, this case of Meissner's might remain an exceptional one.

All of the other publications on the subject are still unsatisfactory because they are all lacking either in convincing bacteriologic data or in reliable clinical data. We shall not discuss them at length. I must, however, quote the most dramatic one, described by Melnotte (17), because it concerns a case of meningitis and because the strain isolated was resistant *in vitro* both to isoniazid and to streptomycin, and treatment with these agents was ineffective. I shall also refer to the most important contribution of Chaves and his collaborators in New York (18), who titrated with a very good technique 385 sputum specimens of previously untreated patients. They describe at least three strains, totally resistant to 5 or 1 $\gamma$ of isoniazid per ml. Unfortunately, the authors do not give us any clinical data, nor do they offer us the assurance that these three cases were not already treated patients who might have slipped by error into a total of 385 patients, assumed to be untreated. An error of 1 per cent, in such a vast inquiry, is, after all, quite permissible and, in a personal communication, Dr. Chaves was so kind as to agree with this remark.

This example is given to show that, at the present stage of research, it is more fruitful to give a complete description of a few selected cases than to know the exact percentage of possibly debatable cases.

* * *

*In conclusion*, when sputum seems to contain only isoniazid-resistant bacilli, it is impossible to assert, even with elaborate *in vitro* tests, that some virulent bacilli (isoniazid resistant or, more frequently, isoniazid susceptible) are not present in the sputum along with the bulk of attenuated ones. Neither is it clearly demonstrated that the attenuated bacilli, whose pathogenicity for guinea pigs is extremely variable, are always harmless for those associated with the patients who excrete them. In these circumstances, therefore, the clinician must first endeavor to obtain—by adequate combination chemotherapy—the disappearance of the tubercle bacilli from the sputum before isoniazid resistance appears. If he fails, he must try to eradicate the responsible focus. In any case, he

must not leave the patient who excretes bacilli, attenuated or not, in contact with unvaccinated persons.

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## GENERAL DISCUSSION

DR. MIDDLEBROOK: Dr. Dubos will not be here, unfortunately, after a few more hours. Dr. Debré and his associates have presented some material in writing which seems sufficiently interesting to justify publication in the proceedings of this symposium, as being read only by title, with a summary by Dr. Dubos. I am sure Dr. Dubos will have more to say than simply to summarize this paper, read by title only; but let him do as he will.

DR. DUBOS: As Dr. Middlebrook told you, Professor Debré has asked me to summarize for you some observations made in his clinic by Dr. Henriette Noufflard, whom many of you know. These observations are summarized in several slides. I shall call for each of the slides in succession, and try to summarize from Dr. Noufflard's notes what their conclusions are.

The accompanying figures concern a study of the susceptibility of tubercle bacilli to streptomycin, to PAS, and to isoniazid, with special reference to tubercle bacilli from primary tuberculosis in children.

First, may I say that these tests were carried out by subcultures in Tween®-albumin medium, and Dr. Noufflard was mindful of the claims that isoniazid can be unstable in that medium. Accordingly, she first carried out this study to see what the expected resistance or susceptibility of a standard strain like H37Rv would be in this medium over a long period of time.

A few strains isolated from pulmonary tuberculosis in adults and older children have been excluded from this study; they had been studied in this laboratory because of their known or strongly suspected resistance, and represent a selection. All of the other strains were isolated from various localizations of primary tuberculosis in children, mainly cerebrospinal fluid from patients with meningitis and gastric washings from patients with pulmonary and mediastinal primary tuberculosis, and a few from miscellaneous localizations (pleural fluid, adenitis, sinuses, pathologic specimens).

*Technique:* The technique has been, with minor variations, the same for all three antituberculous drugs—a dilution method in Dubos liquid medium without Tween 80, at pH 6.8 to 7. Primary isolation was carried out on Löwenstein-Jensen medium; subcultures were made in liquid Dubos medium containing Tween 80.

When such cultures were six to ten days old their turbidity was adjusted, comparatively to a scale made up with BCG (Noël Rist's technique) so as to get an inoculum of approximately 0.03 mg. per 3 ml. of medium. The results were reported after one week of incubation at 37°C. and were read again after two weeks. A control strain (H37Rv) was included in each test comparatively with patients' strains.

As isoniazid has been said to be unstable in Dubos medium, it seems worth while to report the inhibitory concentrations that have been repeatedly found for the control strain.

FIG. 1. Susceptibility to isoniazid. Number of experiments in which the control strain H37Rv has been inhibited by isoniazid.

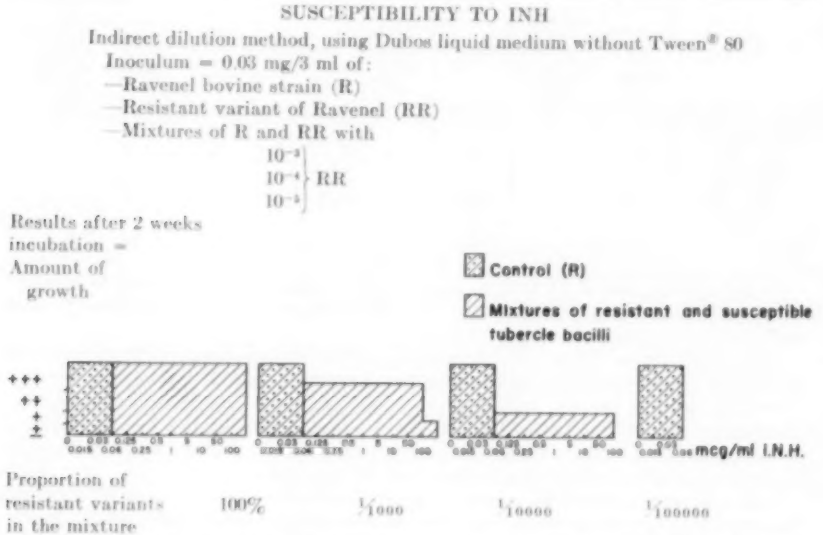
| | Mcg/ml of INH | | | |
|---|---|---|---|---|
| | >0.015 | 0.015–0.03 | 0.03–0.06 | 0.06–0.125 |
| Before August 1954 | 14 | 10 | 5 | 4 |
| Since August 1954 | 17 | 41 | 7 | 0 |
| Total | 31 | 51 | 12 | 4 |

(Dubos liquid medium without Tween® 80, inoculum about 0.01 mg/ml. Results after 2 weeks at 37° C).

Conclusions: In spite of the instability of INH in Dubos medium, H37Rv is regularly inhibited by low concentrations of INH (0.03 or 0.06 mcg/ml).

Because of slight variations from one experiment to another, the routine use of a control strain adds much to the accuracy of the susceptibility tests.

FIG. 2. Susceptibility to isoniazid. Indirect dilution method, using Dubos liquid medium without Tween® 80.




Conclusion: This method allows the detection of 1/10000 resistant tubercle bacilli in mixed populations.

It will be seen from figure 1 that, except in four of the early experiments, H37Rv was always inhibited by isoniazid concentrations of 0.06 γ and nearly always by 0.03 γ per ml. Because of these slight variations, the routine use of a standard strain has appeared to add much to the accuracy of the drug-susceptibility tests.

The experiment reported in figure 2 was designed to find out what proportions of resistant organisms would be detected by the routine technique in mixed populations. It was thus determined that, under the usual conditions, one would report "partial" resistance for one resistant organism out of 10,000 but not for one out of 100,000.

As resistance to isoniazid has been shown not to be always maintained through subcultures, direct susceptibility tests, by a slide-culture method, have been used each time the specimen was rich enough in acid-fast organisms. This hardly ever happened with specimens from primary tuberculosis in children, and we have found no way to overcome this possible source of error.

When a quick answer was needed, especially in cases of meningitis, the pathologic specimens were inoculated directly into mice, and susceptibility tests were made on the organs by a culture technique recently described in the AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES. The results have always been checked by the usual indirect dilution method. No discrepancies have been found as yet.

*Results*[1]

*Susceptibility to streptomycin:* In figure 3 are given the results for forty-eight strains from the cerebrospinal fluid of tuberculous meningitis, eighty-three strains from pulmonary and

[1] The reason for the decreasing number of tubercle bacilli from patients with meningitis

FIG. 3. Susceptibility to streptomycin.

| Years | Tuberculous meningitis | | Primary tuberculosis | | Pleural effusion | | Adenitis | |
|---|---|---|---|---|---|---|---|---|
| | Number of patients with tubercle bacilli | Number having strain resistant to strepto-mycin | Number of patients | Number with resistant strain | Number of patients | Number with resistant strain | Number of patients | Number with resistant strain |
| Sept. 1952 to Jan. 1953 | 23 | 0 | 6 | 0 | 0 | 0 | 0 | 0 |
| 1953 and 1954 and | 18 | 1* | 29 | 0 | 3 | 0 | 6 | 0 |
| 1955 | 7 | 0 | 48 | 1† | 1 | 0 | 4 | 0 |
| Total | 48 | 1 | 83 | 1 | 4 | 0 | 10 | 0 |

* Partially resistant to 2.5 mcg/ml ($H_{20}Rv \times 20$). Medical student, probably contaminated in hospital several years before. Had never received any streptomycin. Treated with INH-PAS alone. Recovered.

† Resistant to over 1000 mcg/ml. Had never received any streptomycin. Same resistance of tubercle bacilli from contaminator's sputum. Treated with INH-PAS. Is doing well.

mediastinal primary tuberculosis (eighty from gastric contents, two from mediastinal lymph nodes found at operation or autopsy, and one from a granuloma removed by bronchoscopy), four strains from pleural fluid, and ten from adenitis.

Two strains were found which were at least twenty times more resistant to streptomycin than was the H37Rv strain. Both came from patients who had never received streptomycin. It is remarkable that no streptomycin-resistant strains have been recovered from treated patients in contrast with what had been found in previous years in tuberculous meningitis treated with streptomycin. This is probably related to the fact that almost all patients since 1952 have been treated with isoniazid-PAS, either in association with streptomycin (in some cases of meningitis) or alone.

One of these two streptomycin-resistant strains was isolated from the cerebrospinal fluid of a medical student who had converted his tuberculin tests several years before, probably after a hospital contamination. He had never received any streptomycin. The meningitis was treated from the beginning by isoniazid-PAS alone. He was doing well when the result of the susceptibility tests was known. He has completely recovered now.

The other streptomycin-resistant strain was recovered from the gastric contents of a child with primary tuberculosis who was getting isoniazid-PAS and was doing well. It was resistant to 1,000 $\gamma$ of streptomycin per ml. The tubercle bacilli in his sputum were also resistant to 1,000 $\gamma$ of streptomycin per ml.

In figure 4 the proportion of strains resistant to streptomycin in untreated patients is indicated year by year. Before 1952, among the strains isolated from tuberculous meningitis, the frequency had seemed to increase: one in 1949, one in 1950, 5 in 1951, 2 in 1952 (making six of the last 109 strains from untreated patients with meningitis). Since then, instead of increasing steadily, they seem to have the opposite trend: none in 1953, one in 1954, one between January and October, 1955 (making two of 145 strains isolated in untreated patients with various localizations of primary tuberculosis).

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is the fact that we get fewer and fewer patients from the beginning of their meningitis. Most have already been treated elsewhere. The reasons for the increasing number of tubercle bacilli from gastric contents are: (*1*) many more cultures of gastric contents have been made since the beginning of the "enquête" of the Institut National d'Hygiène on the treatment of primary tuberculosis; (*2*) the results are much better (fewer contaminants, more positive) since we started using the digestion technique with "Teepol®," described by Tison.

FIG. 4. Susceptibility to streptomycin. Frequency of strains resistant to streptomycin (at least twenty times more than H37Rv) in patients never treated with streptomycin.

| | |
|---|---|
| 1949 = 1<br>1950 = 1<br>1951 = 5<br>1952 = 2 | 8 out of 109 studied (tuberculous meningitis) |
| 1953 = 0<br>1954 = 1*<br>1955 = 1 | 2 out of 145 studied (tuberculous meningitis + other forms of primary tuberculosis) |

* Contaminated several years before.

Conclusion: The frequency seems rather to decrease.

FIG. 5. Susceptibility to isoniazid.

| Years | Tuberculous meningitis | | Primary tuberculosis | | Pleural effusion | | Adenitis | |
|---|---|---|---|---|---|---|---|---|
| | Number of patients with tubercle bacilli | Number having strain resistant to INH* | Number of patients | Number with resistant strain | Number of patients | Number with resistant strain | Number of patients | Number with resistant strain |
| 1953 | 45 | 1 | 11 | 0 | 3 | 0 | 0 | 0 |
| 1954 | 19 | 0 | 28 | 1 | 2 | 0 | 6 | 1 |
| 1955 | 7 | 0 | 49 | 2 | 1 | 0 | 4 | 0 |
| Total | 71 | 1 | 88 | 3 | 6 | 0 | 10 | 1 |

* Resistant to 1 mcg/ml of INH or more.

4 of the 5 resistant strains have been isolated in patients treated with INH from one month to one year. The fifth (from adenitis), partially resistant, was a bovine strain.

Conclusion: Of 180 children with primary tuberculosis, none had tubercle bacilli resistant to INH before treatment (except for 1 bovine strain?).

*Susceptibility to PAS:* The susceptibility of these strains to PAS has also been studied. While among strains from pulmonary tuberculosis resistance to PAS has been a frequent finding, we have found only one strain resistant to PAS in children with primary tuberculosis. This strain was isolated from a patient who had been treated for eleven and one half months with isoniazid-PAS and was resistant to isoniazid as well.

*Susceptibility to isoniazid:* It may be seen in figure 5 that, among 180 children with various localizations of primary tuberculosis, five strains resistant to at least 1 $\gamma$ of isoniazid per ml. have been found. One was found in an untreated patient. It was resistant to 2 $\gamma$ per ml. and partially to 10 $\gamma$ per ml. It was isolated from the pus of a patient with adenitis and was identified as a bovine strain (pathogenicity for the rabbit). The child was living in the country, and no human source of contamination was found.

The other resistant strains were all isolated from patients previously treated with isoniazid-PAS from one month to one year: one in a case of tuberculous mengingitis (the child, treated with streptomycin as well, recovered), and three in primary tuberculosis.

In figure 6 it may be seen at what stage of the treatment these resistant strains have been found.

The catalase activity of 102 strains, studied with the technique described by Middlebrook, is shown in figure 7. Eighty-eight strains susceptible to isoniazid were all catalase positive, while ten of fourteen resistant strains were catalase negative. The four remaining strains may have been mixed populations with respect to susceptiblity to isoniazid.

We can conclude that, but for one bovine strain isolated from an adenitis and resistant

FIG. 6. Susceptibility to isoniazid. Tubercle bacilli isolated from gastric contents in primary tuberculosis under treatment with isoniazid-PAS.

| | Duration of treatment (months) | | | | | | |
|---|---|---|---|---|---|---|---|
| | <1 (1 week to 1 month) | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | ≥6 |
| Number of patients with positive gastric contents | 30 | 18 | 6 | 1* | 0 | 0 | 4† |
| Number with resistant tubercle bacilli | 0 | 2 | 0 | 0 | 0 | 0 | 1 |

* Isolated from granuloma removed by bronchoscopy.

† 2 of these 4 strains have been found at the beginning of a relapse, after the treatment had been discontinued.

Conclusion: 3 strains resistant to INH out of 59 isolated under treatment with INH-PAS in primary tuberculosis.

FIG. 7. Catalase activity and susceptibility to isoniazid.

| Number of strains tested for catalase activity | Catalase activity | |
|---|---|---|
| | + | − |
| Susceptible to INH 88 | 88 | 0 |
| Resistant to INH 14 | 4* | 10† |

* Resistant to: 5-10, 25-50, 2-5, 1-10 } mcg/ml INH

† 4 of these were shown to have decreased pathogenicity for mice, and a fifth (not homogeneous) full virulence.

to isoniazid, we have never seen a strain resistant to isoniazid in a patient who had never received any isoniazid.

DR. MIDDLEBROOK: I would like to call first upon Dr. Schmidt, from Cincinnati, to discuss this paper as well as the paper by Dr. Rist.

DR. SCHMIDT: I would like to say, first of all, that, in general, the observations which we have made on the pathogenicity of isoniazid-resistant tubercle bacilli are in remarkably good agreement with those which Dr. Rist has reported, with a few notable exceptions. It is remarkable that this agreement is so close, in view of the fact that we worked with two entirely different animal species—guinea pigs and rhesus monkeys—and with two entirely different routes of infection.

Our experience has been acquired in simian tuberculosis. It has been acquired largely as a collaborative effort with Dr. Middlebrook, Dr. Lincoln, and others of the Fitzsimons Army group here, and with Dr. Steenken of the Trudeau Laboratories, Trudeau, New York.

Our studies have included experience with twenty-three isoniazid-resistant strains which were catalase negative and which were able to grow as well on 7H9 medium in the presence of 10 $\gamma$ of isoniazid per ml. as on control medium without isoniazid. In addition, we have had experience with some six other isoniazid-resistant strains which had diminished, but not complete, absence of catalase activity and grew on isoniazid concentrations of 0.1 $\gamma$ to 1.0 $\gamma$ of isoniazid per ml. in this same medium just as well as they did on the control medium.

I would like to point out first the experience we have had with the organisms which are catalase negative and resistant to 10 γ of isoniazid. The range in pathogenicity among these strains begins with the type of response which one sees with BCG following intratracheal inoculation of massive numbers of these organisms; and by massive numbers I mean inocula in the neighborhood of 50 to 100 million bacilli.

In such cases there is no roentgenographic evidence of pulmonary infiltration at any time up to three months after inoculation, and no evidence of residua of active infection when the animals are sacrificed three months after inoculation.

This is the bottom end of the spectrum. The other end of the spectrum includes the production of a tremendous amount of pulmonary infiltrate and sometimes such a terrific amount of involvement that one has very extensive excavation of the involved area, followed by death. I am not certain whether the rare deaths which have been observed have actually been due to progressive tuberculous infection itself or to early loss of pulmonary function.

If one were ignorant of the pathogenicity of isoniazid-susceptible organisms, it might be inferred that our experience covered the total range between the pathogenicity of isoniazid-susceptible bacilli and BCG; but, in reality, this is not the case, because if one titrates the numbers of living bacilli required to produce significant infection, one finds that with any of the wild type of susceptible strains (29 strains) an inoculum of 100 or more organisms will produce, within three months, an overwhelming infection in the monkey. Even with the least attenuated catalase-negative strains resistant to 10 γ of isoniazid, an inoculum of 100,000 or more bacilli is necessary to accomplish the same end result.

The organisms which have lesser degrees of resistance to isoniazid and some catalase activity show a very mixed picture, varying all the way from a response similar to the median of the catalase-negative group up to almost complete pathogenicity, in which an inoculum of 10 to 100 organisms will produce fatal, overwhelming disease.

Dr. Middlebrook: Dr. Mitchison, since your paper will not be concerned primarily with this problem and you have had much experience, we would like to hear from you.

Dr. Mitchison: There seems to me a matter of particular importance raised by Dr. Rist and Dr. Schmidt's discussions, and that is whether there is anything qualitatively different in the nature of the loss of pathogenicity in isoniazid-resistant strains, as compared to a similar loss that might be obtained, say, by repeated passing of fully virulent organisms in artificial culture media.

The range of loss of pathogenicity in isoniazid-resistant strains varies from a degree about equal to, or perhaps slightly less than, that found with BCG, to a degree which is pretty well fully virulent. This range of attenuation is one that has hardly been studied at all in organisms other than isoniazid-resistant strains.

What would seem to be required is a more detailed study in different animal species of strains of bacilli susceptible to isoniazid, but attenuated by passage in artificial media to a degree less than that of BCG, and to compare such strains with isoniazid-resistant strains to see whether there are qualitative as well as quantitative differences.

Dr. McDermott: If I may make a comment with reference to the very complicated nature of this situation which Dr. Rist has so ably presented, and which has been discussed with so much information, the particular point I would like to direct myself to is Dr. Dubos's citation of Dr. Noufflard's 1 in 10,000 cells detectable, that is, an isoniazid-resistant cell that would require only 1 in 10,000 to give one a standard reading of isoniazid resistance for that particular culture.

In some work in mice, along the lines which Dr. Mitchison has done, Dr. Tompsett has studied (with very careful population studies) these isoniazid-resistant strains. These strains have not been subjected to the catalase test; nevertheless, the precise numbers of organisms known to be resistant or susceptible to varying degrees of isoniazid are known and are documented.

In that situation, in the mouse, one can get isoniazid-resistant strains which multiply *in vivo*, in mice, equally well with the H37Rv. Then (and this is the point of my remarks) one can take strains which have percentages of isoniazid-resistant cells, and it takes a

remarkably low percentage, of the order perhaps of only 5 per cent or less of cells (even down to 1 per cent) resistant to isoniazid to show some degree of loss of isoniazid usefulness in that particular situation.

So, we are almost in the same situation as the national pictorial magazines, where one magazine will run an ad that they have the most advertising dollar-wise, and another will run an ad that they have the most advertising page-wise. Let us not be deluded by any particular one of these citations, but remember that they all have to be put together.

Dr. Gertrude Weiss (Denver, Colorado): I would like to address two questions to Dr. Raffel. One concerns the patients who showed tuberculin hypersensitivity in the absence of gamma globulin. Were these patients investigated for the presence of alpha globulins? I think, if alpha globulins were found, this would be an important thing in evaluating the whole situation.

I have a second question. I would like to ask Dr. Raffel to make a brief comment on the correlation or lack of correlation between the hypersensitivity *in vivo* and hypersensitivity as demonstrated in tissue culture.

Dr. Sidney Raffel (Stanford, California): The first question concerned the aglobulinemic patients. I have received only two personal communications on this which are fairly detailed, and I must speak from the general reports concerned with agammaglobulinemia rather than from these two particular instances.

The plasma protein picture is apparently completely a normal one in patients with agammaglobulinemia, with the one exception that they lack gamma globulin. Consequently, apparently they all have alpha and beta globulin in full measure.

Since the two gentlemen who wrote these letters to me, describing their patients, made no reference to the fact that these patients differed in any respect from other patients with agammaglobulinemia, I presume they did have alpha globulins.

That is a very good point, and it is one that I also considered. In the work with respect to this peculiar antibody eventuating as the one responsible for tuberculoprotein allergy, then obviously an alpha globulin correlation of this kind is of utmost importance.

The second question had to do with correlation between delayed type of hypersensitivity and that demonstrated in tissue culture. I am among those who believe that this type of hypersensitivity is demonstrable with isolated cells, or in isolated situations in the body where there is no blood supply, as in the cornea, because, whatever the eventual mechanism of origin of the hypersensitive state may turn out to be, individual cells appear to be directly responsive to antigens. This is apparently not the case in the, broadly speaking, anaphylactic types of hypersensitivity.

Consequently, in our own work on many occasions we have employed tissue culture as an objective criterion of whether a state of delayed hypersensitivity exists. Others have done this, too. I know, on the other hand, that there are objectors to this viewpoint. There are workers who have failed to find this correlation, but we have been quite successful with it, and a number of other people have also been successful with it.

I am not sure that this is what you have in mind in the way of an answer.

Dr. Hattie Alexander (New York, New York): I would like to ask Dr. Raffel a question, if I may. If development of hypersensitivity to tuberculin is the result of a protein and the bacillary lipid, both these substances must be in tuberculin. Why do we not get hypersensitivity as a result of repetition of tuberculin tests with OT—or is that a misinterpretation?

Dr. Raffel: It is a perfectly valid question. Actually, I dobut that there is much, if any, of the lipopolysaccharide in OT. However, there may be. Certainly there is protein of a kind, but—

Dr. Middlebrook: You mean polysaccharide and lipid.

Dr. Raffel: Dr. Alexander was talking about the two elements which are concerned in the induction of a hypersensitive state, which would be the lipopolysaccharide plus the protein.

Dr. Middlebrook: Did you mean lipoprotein or polysaccharide?

DR. RAFFEL: I said I thought there was practically no (or nearly no) lipopolysaccharide material present in this watery solution. There is protein of a kind. I want to qualify the "of a kind." The tuberculin, of course has been quite drastically treated. If it is Old Tuberculin, it has been heated in a boiling water bath for eighteen hours, which is pretty rough treatment for almost any organic substance, especially proteinaceous materials; and so, as the work of Seibert and others has shown, tuberculin contains a series of degraded molecules which can be classified, I suppose, as haptenes, so that they are capable of eliciting the reaction in the infected individual or in a test tube with preformed antibodies, but are not capable of actively inducing the state of reactivity.

DR. DUBOS: Dr. Raffel, I would like to ask you a question concerning the third line that was not on your slide, which you asked us to contribute mentally.

As I remember, that had to do with your own failure to produce immunity by injecting simultaneously lipopolysaccharide and protein, a technique which elicits tuberculin hypersensitiveness.

You made the statement that you could not obtain immunity by that particular technique. However, as I understood you, you qualified your statement, indicating that some others would not agree with you. You seemed to indicate that there are investigators who claim that they can achieve immunity by that technique. I do not know of any such. Would you mind giving us more information?

DR. RAFFEL: Yes. In fact, the references appear in the course of the paper, but I didn't mention specific names. In this case, Dr. Choucroun, I think, is the foremost proponent of the idea that a lipopolysaccharide of the organism, I believe without the protein, can immunize. Perhaps the protein is necessary. I am sorry, but that point eludes me. Emanuel Suter, at Harvard, has also indirect evidence that this may be so, not directly in the animal, not by challenging animals which have received these components, but rather by examining the behavior of macrophages of such animals in the tissue culture toward living tubercle bacilli and finding that the macrophages of animals which have received lipopolysaccharide alone appear to suppress the intracellular growth of the bacilli.

Oddly enough, the macrophages of animals which have received both components, lipopolysaccharide and protein, do not have this inhibitory property. Those are the two outstanding proponents of this viewpoint that I can think of at the moment.

DR. DUBOS: In the case of Dr. Choucroun you refer to statements, not experiments.

DR. RAFFEL: Perhaps you are right.

DR. CANETTI: I would like to add a word to the question. First, a technical point. Choucroun tries to immunize with lipopolysaccharide alone and, in the second place, I think her main work is going on now, and she does it just on children. She does not do the work on guinea pigs. So, it is not very easy to show if immunity has been achieved or not; yet, as Dr. Raffel said, she is one of those who believe that these lipopolysaccharides can confer a certain amount of immunity.

May I ask a few questions on Dr. Rist's paper and on certain implications therein. I think there is an important point which should be considered a little more closely, and that is the question of these cavities which Dr. Rist has observed very lately in some of the guinea pigs inoculated with resistant strains.

You know it is extremely difficult to achieve, in guinea pigs, something of a late type of tuberculosis or an isolated organ tuberculosis resembling what we see in man. That is a very difficult thing. One can, of course, get such kinds of tuberculosis by using vaccinated guinea pigs, or after treating guinea pigs by streptomycin and leaving them to relapse, but it is not easy.

In Dr. Rist's animals, as I understand it, he observed a certain number of instances in which he got that late, isolated type of tuberculosis of the adult, with open cavities in the lung. He said that these cavities were swarming with bacilli and opening to the bronchi.

What I want to know is this: First, did he observe cavities in other organs than the lungs? Second, if he did, did he observe the same amount of bacillary growth in such cavities?

In the literature, there are some observations of a German worker who had some cases

of this type of guinea pig tuberculosis with cavities in the lung in certain animals, and in the spleen in other animals. He merely showed that cavities in the spleen don't show bacilli, or show very few bacilli, whereas cavities in the lungs of the same histologic structure swarm with bacilli as soon as they open into the bronchi.

This clearly points to the favorable influence of oxygen for bacterial growth in cavities.

Dr. Long: After Dr. Rist replies to that question, I wonder if he would include in his reply a statement with regard to the possibility of tubercle bacilli being in the spleen in a nonrecognizable form, at least not in the typical acid-fast form found in the lesions in the lung.

Dr. Rist (Paris, France): The annoying thing in the cases I have just reported is that they are quite like clinical cases; but I am not at all sure I can reproduce the picture at will when I return to Paris. It was only in a few among numerous guinea pigs that I had the chance to obtain those findings.

It is quite true that the cavities in the lungs were all open to the bronchi. As for the other lesions I found in the same animals, I referred to the very considerable hypertrophic tuberculosis in the spleen in which there were many giant cells and few bacilli and no cavities. However, in one of the cases, the first case that I cited, with big cavities in the lung swarming with bacilli, I did not have time to show the spleen photomicrograph. The spleen was full of very large areas of calcification which we may assume to have taken the place of big cavities. I never saw such calcification in the spleen of guinea pigs infected with ordinary strains of tubercle bacilli. It may well be that there had once been a big necrotic focus which healed while the same focus in the lung did not.

Dr. Middlebrook: Just a moment. What do we mean by "cavity"?

Dr. Rist: I was answering Dr. Canetti. He asked me whether I found cavities in other organs. A cavity supposes that something has been eliminated.

Dr. Canetti: Cavities have been obtained in the spleen by some authors.

Dr. Middlebrook: What does the air space open into?

Dr. Canetti: I can't tell you exactly how the evacuation of caseous material happened. It may have been a kind of suppuration.

Dr. Middlebrook: But it contained air?

Dr. Long: I think it is a cold abscess; that's what it amounts to.

Dr. Canetti: I call a cavity a space—

Dr. Middlebrook: A cavity is an abscess?! I think this is important. With regard to these organisms swarming in these cavities, he called them cavities. We don't say that, if a human being has a cold abscess, he has a cavity in his groin.

Dr. Canetti: What I mean by "cavity"—

Dr. Middlebrook: You had better use "cavité," then, because it is not used that way in English.

Dr. Canetti: No; it is the same word. It is just something lacking in a tissue. It is surrounded by a capsule, and that is what has been observed in spleens. I asked Dr. Rist if he has observed anything like that in the spleen, and if he has—

Dr. Middlebrook: Don't you call those "closed necrotic lesions"?

Dr. Canetti: They are devoid of anything in them. There is nothing in them. They are free. You can call them cysts if you want to.

Dr. Terplan: May I say a word on that? I think Dr. Canetti is right, because we see so-called cavitary destruction even in lymph nodes, where massive caseated lymph nodes are eliminated usually by opening into some preformed channels, like the bronchi. As far as the term is concerned, "cavitary destruction of the lymph node" has been used in tuberculosis literature.

Dr. Middlebrook: They have air in them. But how does one evacuate the material from a splenic abscess? By what route does one evacuate the necrotic contents of an abscess in the spleen?

Dr. Long: You can't get a cavity with air in it in the spleen, of course. You can get a cavity that has ceased to have straight caseous material because, if the conditions which

prevent the collapse are such, it will fill up with fluid. You can have that type of thing in the spleen.

I don't know whether you want to call that a cavity or not. I suppose by that time it is a cyst. I think everybody knows what he is being asked, however. The trouble is in the use of the word.

DR. MIDDLEBROOK: I think there is a very important point that Dr. Rist has emphasized, and that is that, in those lesions of the lung in which evacuation has been possible, and air or the bronchiolar gases have been admitted, here he sees, just as one sees in a treated tuberculous patient, massive multiplication of catalase negative tubercle bacilli in *open* pulmonary cavities and, as in man, in the opinion of some investigators, no evidence of progressive tuberculous disease elsewhere.

So, it is at this level that I thought Dr. Rist was discussing the problem. Whether or not there were progressive lesions in the spleen was most important; but whether or not they are "cavities" would seem to be just a semantic problem between you and me, Dr. Canetti.

DR. RIST: I understand that Dr. Canetti wanted me to answer him. I did find many bacilli in the lung cavities, and I did not find many in other lesions in the same animals. That was quite true.

DR. LONG: What about the possibility—

DR. RIST: We often found a spleen, for instance, in which we thought we should find many bacilli on the ordinary smears, and in which we didn't find any; and on culture it was swarming with them. The possibility of nonvisible forms—

DR. LONG: Then we are just about where we used to be on that. Okay!

DR. MIDDLEBROOK: Yes, wherever that is.

# DYNAMICS OF ANTITUBERCULOUS CHEMOTHERAPY[1]

WALSH McDERMOTT,[2] ROBERT M. McCUNE, JR.,[2] AND RALPH TOMPSETT[2]

Any smugness which we might have had concerning our knowledge of tuberculosis and its treatment should have been amply punctured by Professor Ustvedt's marvelous analysis yesterday of the four fatal cases due to BCG.

As you will recall, he cited one patient who died who had drug-susceptible bacilli and who had received the drugs to which the bacilli were susceptible *in vitro*.

In such situations, with our marvelous scientific knowledge, we have to blame something; and, in keeping with that tradition—ever since Jonah and the whale—we blame the least familiar element in the situation. In Professor Ustvedt's case, it was the host. In other words, it was host failure. It could not have been a failure of our drugs, because we know all about them!

The point which the case presented by Professor Ustvedt dramatized was that antimicrobial therapy is a three-way proposition, involving not just a drug-parasite relationship but a drug-parasite-host relationship.

All of us yesterday got up and publicly recounted, if you will, how very little we knew about even the two-way relationship between the parasite and the host. So now, perhaps fleeing from our ignorance in that field, we will complicate the situation further by considering a three-way relationship.

Several years ago, Dr. Robert McCune, Dr. Ralph Tompsett, and I started working in this field, using a technique developed in Dr. Dubos' laboratory by Drs. Frank Fenner, Cynthia Pierce, and Samuel Martin. This is a technique of microbial enumeration with which many of you are familiar.

Our approach was based on the assumption that an essential element in the pathogenicity of a microorganism is its ability to multiply within the host. We thought that the behavior of the microbial census, if you will, under various artificially induced variables—in our instance, drugs—might provide some information on this very highly complicated problem. The basic experimental situation was as follows:

One takes a very large group of mice and infects them intravenously with tubercle bacilli. Then, at various periods of time, one samples this total group of mice, removes various organs (in this case the lung and the spleen), grinds them up, and enumerates by cultural techniques the number of culturable units per milliliter of organ emulsion.

In the figures, the ordinate represents the census of tubercle bacilli in terms of culturable units. The abscissa represents time after infection.

The point seen in figure 1 is something which was noted in Dr. Dubos' laboratory, and is quite important, namely, that populations of tubercle bacilli behave differently in the lung than in the spleen. One can see that in both organs the

[1] As the speaker failed to submit a manuscript for publication, the recording of his presentation is reproduced here.—*The Editor*.

[2] New York Hospital-Cornell Medical Center, New York, New York.

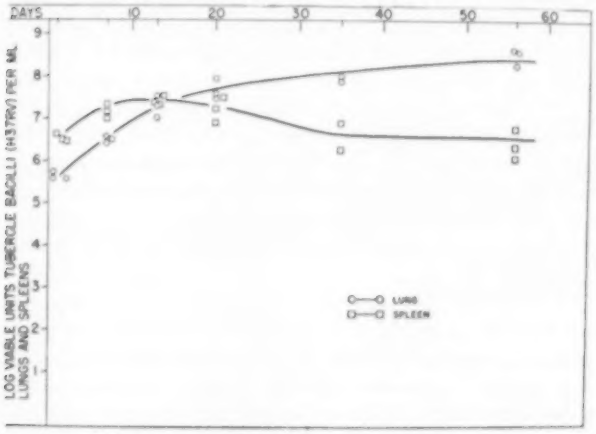


FIG. 1. Microbial populations (H37Rv) in lungs and spleens of untreated mice.

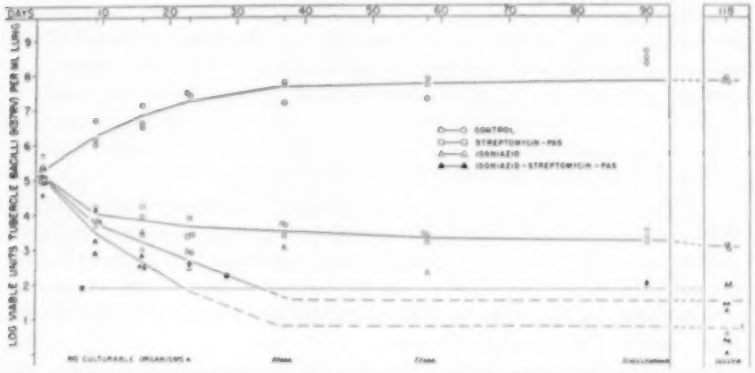


FIG. 2. Influence of drugs on microbial populations (H37Rv) in mouse lungs during sixteen weeks of therapy. Comparative effect of isoniazid, streptomycin-PAS, and isoniazid-streptomycin-PAS.

population of tubercle bacilli at first increases and then stabilizes. In the spleen, once stabilized, it actually falls and then stabilizes again. The important thing is that the behavior of the microbial population is distinctly different in the lung and in the spleen. These two types of behavior hold throughout all of the experiments, showing a difference in the host-parasite relationship in two different organs.

In figure 2 it may be seen that the populations of tubercle bacilli likewise behave differently with respect to drugs, depending upon which organ is involved.

For example, in the lung, one gets a lowering and a stabilization of the population with streptomycin. With isoniazid and isoniazid-PAS and with strepto-

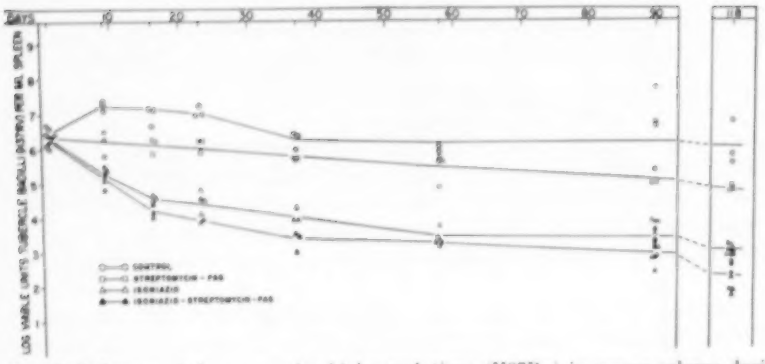


FIG. 3. Influence of drugs on microbial populations (H37Rv) in mouse spleens during sixteen weeks of therapy. Comparative effect of isoniazid, streptomycin-PAS, and isoniazid-streptomycin-PAS.

mycin, one gets a greater lowering; indeed, the dotted lines in the figure and in subsequent figures indicate animals from which no bacilli could be cultured by standard techniques. So, it may be seen that, in the lung, isoniazid alone or isoniazid-streptomycin and PAS may cause the tubercle bacilli to vanish.

In contrast, in the spleen, with these same drugs the bacilli do not vanish. In figure 3 the upper curve is the trend line for the control animals; the middle curve is the microbial census under streptomycin; and the two bottom lines are isoniazid alone and isoniazid along with streptomycin and PAS.

These experiments illustrate one of two basic phenomena which we studied. It is this stabilization of the population, despite continued antimicrobial therapy, that interested us. This is, in effect, *persistence* in the sense that the late Professor Biggers used the term. The tubercle bacilli isolated at these various points (or their descendants, at least, which, of course, are what we test) are not resistant to the drugs used in the conventional sense.

In other words, the descendants of these bacilli (or these bacilli, in crude terms) are drug susceptible. Nevertheless, they remain in the tissues. Moreover, as you will see, they remain there even though there are not many of them. They persist, despite a continued attack with the very drugs to which they are so susceptible when tested *in vitro*.

The problem is quite a complicated one, as may come out in the discussion. For, when ordinary drug-resistant bacilli do emerge to predominance, the population (as it does anywhere else) will increase. This increase, however, is not invariable, which is a subtlety of the situation. It can be stated unequivocally, however, that this phenomenon of persistence has nothing to do with drug resistance in the orthodox sense.

I would like to make two points about the histopathology of these lesions. The first is that it may be impossible to distinguish between the lesions seen with one drug from those seen with another drug even though there may be a two log difference in the microbial census in the two situations.

Thus, the histopathology of considerably different situations may appear identical. In effect, a Colorado ghost town and New York City will look the same under the microscope, yet by the culturing technique one can detect the very gross population differences which exist between them.

The second point I would like to make is something which we all know, but which I think is a rather fascinating one. If you will observe the population curves in the lung, it may be seen that the population does stabilize. The census goes up and then stabilizes, and it remains stable throughout the ensuing months, even though the lesions are progressive and the animal dies of this particular infection in about five or six months.

So, in effect, one may be sending in fresh troops all the time, but one is not using any additional personnel in terms of numbers to have this progressive lesion continue. The microbial census is stable, the lesions progress, and the animal dies.

This stabilization phenomenon was seen, and even more vividly, with the use of hydrocortisone or cortisone in a series of experiments conducted by Dr. John Batten in association with Dr. McCune. The splenic populations still stabilize despite hormone effects, whereas in the lung the census goes right up to very high levels. With the spleen, the population increases. It may increase as much as ten- to fifty-fold; nevertheless, it does eventually stabilize. The animal dies of the pulmonary disease in the process but the stabilization of the population does occur in the spleen.

* * *

This persistence of drug-susceptible bacilli, despite treatment with the appropriate drugs alone or in various pairs and trios, has been found for some nine drugs which have demonstrable antituberculous activity in animals.

In only one situation, namely, with the pyrazinamide-containing regimens, has

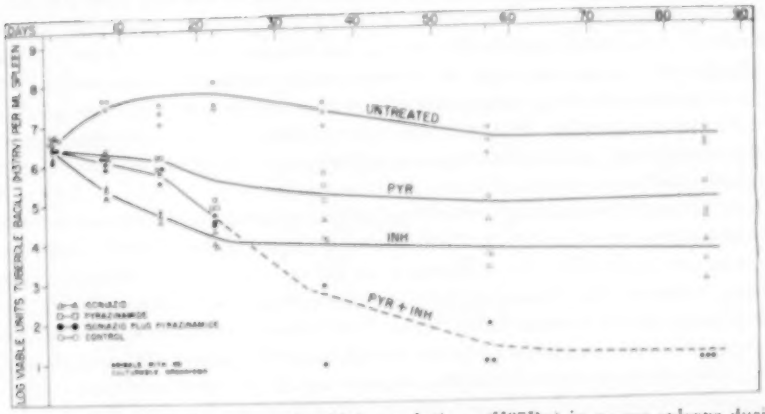


FIG. 4. Influence of drugs on microbial populations (H37Rv) in mouse spleens during eighty-five days of therapy. Comparative effect of isoniazid and pyrazinamide singly and together.

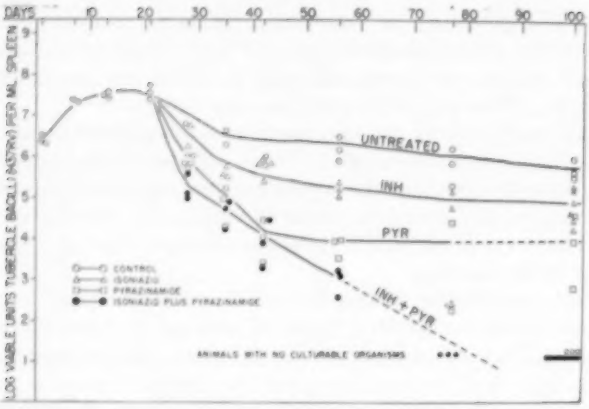


FIG. 5. Influence of drugs on microbial populations (H37Rv) in mouse spleen during seventy-nine days of therapy. Lesions established at start of treatment. Comparative effect of isoniazid and pyrazinamide singly and together.

it been possible to interrupt this persistence phenomenon. During the first two weeks of therapy (figure 4) the pyrazinamide apparently antagonizes the isoniazid; then, very soon thereafter, the population falls rapidly to below the point of detectability. Pyrazinamide, as you all know, is a nicotinamide derivative.

The results in figure 5 simply show that, in an established infection, namely, when the bacilli have been allowed to multiply in the animal for a twenty-one-day period and when grossly visible lesions are present, the pyrazinamide still works with the isoniazid. Indeed, in these circumstances the pyrazinamide alone, as may be seen, is superior to isoniazid alone in terms of reducing the census. In other words, the phenomenon of the vanishing of the tubercle bacilli from the tissues occurs equally well in the twenty-one-day infection as in the zero-day infection, when drug and infection are started simultaneously.

The bacilli thus vanish from the tissues with pyrazinamide-isoniazid in so far as can be determined by microscopy, by culture, by guinea pig inoculation, or by the technique of Dr. Hobby and her associates (that of a very delayed, prolonged incubation *in vitro* and then subculturing). By all of these methods it has been impossible to demonstrate bacilli in the tissues.

In addition, the culturing method has been stepped up so that the entire organ is cultured—a situation which, when one adds known viable bacilli to the organ, permits one to detect with uniformity as few as one or two culturable units. These, however, are "store-bought" tubercle bacilli, and not ones which have been in an animal subjected to a drug. I am trying merely to make the point that by all of the available methods, including every method we know about or have devised, these tubercle bacilli are not there. They have vanished.

Hence, we have a truly latent infection in the sense that it is there—I have gone ahead of myself! Let me say that we cannot show the bacilli are there. Nevertheless, the bacilli are there, or at least they are there in approximately

one-third of the animals because, if we discontinue therapy and wait ninety days, we can then cultivate bacilli from the spleens of approximately one-third of the animals. It varies a little bit and it varies with respect to how much treatment they have had, but not appreciably. So, in approximately one-third of the animals at a ninety-day period after treatment is stopped, bacilli can again be cultivated.

Those bacilli, too, in so far as the point has been studied, are susceptible to isoniazid and pyrazinamide *in vitro*. At least, they are definitely susceptible to isoniazid. The number of strains studied for pyrazinamide susceptibility is only some eight or nine.

Hence, we have what we like to regard as a truly *latent* infection in that the infection is there but is hidden beyond diagnostic reach. We contrast this with what we call a *dormant* infection which is the type of situation one gets with all of the other drugs—when the living bacilli are readily demonstrable. The dormant infection is not active, the populations are not increasing; nothing much is happening; nevertheless, it is not hidden beyond diagnostic reach as in latent infection.

One possibility, which is an alternative situation to the sort of thing Dr. Long mentioned a few minutes ago, but is in the same vein, may be occurring. Could it be that these tubercle bacilli which are not only invisible are merely not culturable?

It would take really quite a microbial census to appear by microscopy. If you suddenly had a widespread alteration of the tubercle bacilli, whereby they could not be cultured, and if the census were just below the limits of detection by microscopy (and that still is an awfully big population), we might be deluding ourselves in believing in these drastic population declines.

Be that as it may, whatever happens is unique to the pyrazinamide-containing regimens, and not to the others.

The other type of studies which we have done on this particular point have to do with time-dose relationships, and in these it has been shown that the pyrazinamide must be administered for at least eight to twelve weeks. The particular dose which we have used in our studies is 2 per cent in the diet, which is a "whopping" dose. In the time-dose studies it was shown that this could be reduced by approximately 75 per cent, which still represents a big dose; but then, of course, we were using very large doses of streptomycin and isoniazid as well. Moreover, there is considerable evidence that pyrazinamide effects in humans can be produced in considerably lower doses than is the case in the dog and in the mouse.

We then studied the respective roles of pyrazinamide and isoniazid. Pyrazinamide alone occasionally results in this latency phenomenon in the mouse spleen. Indeed, it does this more often than not in the twenty-one-day infection.

The one nonuniform situation of this entire series of experiments is that, when pyrazinamide is used alone, we are never entirely certain what will happen. Sometimes it behaves like isoniazid alone; sometimes there is a drop in population and then a resurgence of growth after forty-two days or so; and sometimes,

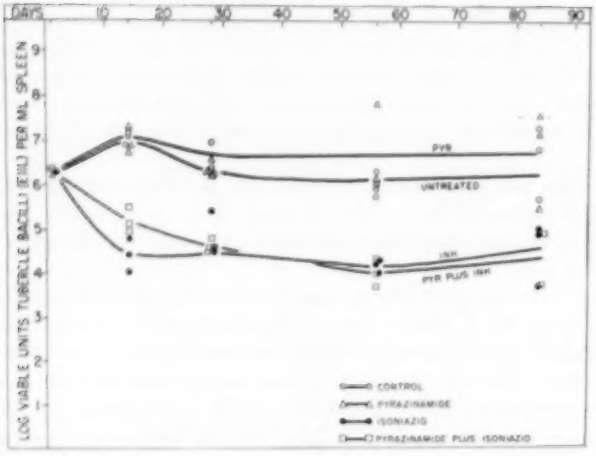


FIG. 6. Effect of drugs on population of pyrazinamide-resistant tubercle bacilli (E 11 L) in mouse spleen.

particularly in the twenty-one-day infections, there is complete vanishing of the bacilli in perhaps one-half of the animals. However, we cannot uniformly produce the vanishing phenomenon with pyrazinamide alone.

An "antagonism" of isoniazid by pyrazinamide made it seem rather likely that both drugs play a specific role in this process. In figure 6 one can see the lack of effect of pyrazinamide on a culture derived from an animal in which the tubercle bacilli had started to re-multiply under pyrazinamide therapy—in other words, a truly pyrazinamide-resistant organism. (These experiments were performed by Dr. Wasz-Höckert of the University of Helsinki who has been working in our laboratory during the last year.)

In figure 7 may be seen the same thing with an isoniazid-resistant strain, namely, although at times a few isolated observations showed the vanishing phenomenon; nevertheless, at the end of the experiment, when we used very large numbers of animals to prove the point, it was possible to cultivate the drug-resistant organisms from the spleen.

What I am saying is that this particular strain of isoniazid-resistant tubercle bacilli was not handled in any unusual way by pyrazinamide, especially in the lung.

Thus, we had reason to believe, particularly because of the antagonism phenomenon, that both drugs played roles and some sort of specific roles. The next thing was to try other drugs, and it became immediately apparent that such diverse substances, chemically, as streptomycin, PAS, oxytetracycline, all worked just as well as isoniazid in producing this phenomenon with pyrazinamide.

The fact that pyrazinamide alone sometimes worked by itself, so to speak, with the vanishing phenomenon, and the fact that any companion drug worked

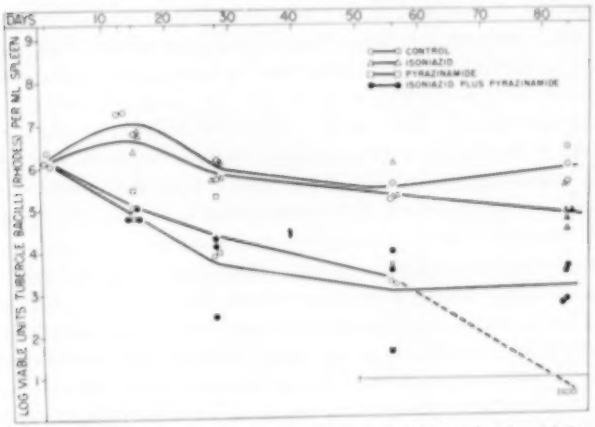


FIG. 7. Influence of drugs on isoniazid-resistant (>0.25 <0.5) microbial populations in mouse spleens during twelve weeks of therapy. Comparative effect of isoniazid and pyrazinamide singly and together.

with it, made it appear likely that what happened was simply that the population was reduced by the pyrazinamide to a low point, and then the companion drug simply suppressed a very small number of tubercle bacilli for the remainder of the experimental period. Indeed, in such circumstances (and we were down perhaps to fewer than 10 organisms per spleen), they might die off. You might sometimes have an eradicative phenomenon and sometimes not.

Accordingly, we studied this point in a series of sequential experiments. The notion that the pyrazinamide was the heavy drug in the team which dropped the population down to a low point was particularly good because of the fact that, when pyrazinamide was used alone, one would get re-multiplication of some strains and re-multiplication from a low census rather than re-multiplication from a high census. So, it looked as if we were dealing with mixed populations, a few members of which were pyrazinamide-resistant from the beginning; and that, if the great mass of the population was reduced with pyrazinamide, another drug could take care of the orthodox pyrazinamide-resistant tubercle bacilli.

This seemed not too unlikely either, because of the fact that strains of bovine origin are completely nonsusceptible to pyrazinamide—a phenomenon which gives food for further speculation which I shall not go into at the moment.

In any event, one working hypothesis was that the pyrazinamide reduced the population to a low level and the companion drug then took over.

The sequential experiments, however, threw some sand into the gears of that particular hypothesis. We used four weeks of one drug and eight weeks of the other drug sequentially, ringing all of the obvious changes; that is, eight weeks of pyrazinamide and four weeks of isoniazid, and so on.

In the first place, the fact established previously—that it took at least eight weeks of pyrazinamide to produce the phenomenon—again showed up. In other

words, in none of the experiments in which only four weeks of pyrazinamide was given did latency occur. Furthermore, to our great surprise, only one of these four regimens was effective in producing the vanishing phenomenon; and it was the regimen in which isoniazid was given for four weeks, followed by pyrazinamide for eight weeks. In other words, eight weeks of pyrazinamide starting at the beginning, even though it was followed by four weeks of isoniazid, didn't work at all; but, if we started the pyrazinamide after *four* weeks of isoniazid, then the vanishing phenomenon did indeed occur.

* * *

There are several side aspects of this particular study by Drs. McCune, Tompsett, and myself which I shall not discuss here. One is whether it is the lesion or the bacilli which are primarily concerned in the maximal activity of the pyrazinamide. One can argue that the lesion matures and permits the pyrazinamide to work, because it does work *in vitro* only in acidic environments and it does work intracellularly; or, one can argue that some alteration in the bacilli themselves is necessary. Be that as it may, the only things that can be said about the pyrazinamide-isoniazid phenomenon in the experimental situation are, first, that it takes eight weeks of pyrazinamide in a total period of twelve weeks of chemotherapy; and, second, that the bacilli have to be altered in some way (that is too strong a statement)—that the pyrazinamide must either be accompanied or preceded by another antituberculous companion drug.

When one realizes that, even though the bacilli vanish and there is present what we can call a truly latent infection, the bacilli are, nevertheless, still there in these particular time-dose relationships after ninety days and that the organisms are still drug susceptible, I think you will agree with me that it shows that "you can't win."

# THE BACTERICIDAL ACTIVITIES OF ANTITUBERCULOUS DRUGS

D. A. MITCHISON[1] AND J. B. SELKON[1]

From the historical point of view, the chemotherapy of tuberculosis has presented two main problems. The first of these is the rapid emergence of drug-resistant tubercle bacilli during treatment. This problem has largely been solved by the simultaneous use of two chemotherapeutic drugs, although many failures of treatment still occur in individual patients, mainly as a result of earlier courses of therapy with single drugs or because of inadequate or intermittent dosage.

The second problem is concerned with the length of treatment necessary to lower the number of viable bacilli to an extent sufficient to allow the successful operation of the host's defense mechanisms, or possibly even to achieve their complete eradication.

It is remarkable that *in vitro* a culture of tubercle bacilli in the lag phase of growth can be sterilized in about two to four weeks by two drugs present in concentrations that would be expected in the lesions; whereas many authorities consider that courses of chemotherapy in human tuberculosis should last from six months to three years to avoid the risk of relapse.

The ability of tubercle bacilli, still susceptible to the drugs, to persist for long periods in the lesions of patients undergoing chemotherapy can be explained on two hypotheses. One of these assumes that there are certain sites within the body where the drugs do not penetrate or are inactivated. For reasons discussed elsewhere (1), this hypothesis is thought to be unlikely, except that bacilli may perhaps persist in solid caseous lesions or for short periods inside cells out of the reach of streptomycin and PAS. An alternative and more probable hypothesis is that the bacilli persist because their metabolism is inactive and they are, therefore, not killed by the chemotherapeutic drugs.

The work to be described was carried out with the aim of investigating the effect of various agents or conditions which might inhibit the metabolic activity of tubercle bacilli on the bactericidal powers of drugs such as isoniazid and streptomycin. The suspension of starved tubercle bacilli in non-nutrient medium has been shown to inhibit the bactericidal action of streptomycin (2) and of isoniazid (3) on these organisms. Furthermore, oxytetracycline in bacteriostatic concentrations has been found to prevent or slow down the bactericidal activities of isoniazid, streptomycin, and viomycin (4, 5). However, both complete starvation and the presence of oxytetracycline may present conditions to the bacilli which are dissimilar to those produced by the defense reactions of the animal or human host.

An attempt has also been made to determine whether previous BCG vaccination in guinea pigs influences the bactericidal activity of isoniazid, since it is possible that the immune process may be principally either bacteriostatic or

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bactericidal and may therefore act antagonistically or synergistically with a bactericidal drug.

## METHODS

The *in vitro* studies were carried out with the H37Rv strain of *M. tuberculosis* and a series of control experiments was also set up with strains of *Staphylococcus aureus* and *E. coli*. *In vivo* work was carried out with an attenuated variant of the Branch bovine strain of *M. tuberculosis*, which was capable of causing markedly delayed death of guinea pigs. The latter strain was chosen so as to obtain a host-parasite relationship in guinea pigs as nearly in equilibrium as possible.

Viable counts were carried out as deep counts in the 7H 3 medium described by Cohn, Oda, Kovitz, and Middlebrook (6), solidified in thin layers in screw-capped "medicine flat" bottles by the addition of a silica sol. Readings were made after four weeks' incubation.

In the *in vivo* experiment, guinea pigs were vaccinated intramuscularly with 1 mg. (wet weight) of the Copenhagen BCG strain seven weeks before challenge. The challenge infection, with the Branch attenuated strain, was given to two groups of guinea pigs (either previously vaccinated or unvaccinated) by the intravenous route in a dose of $Log_{10}$ 6.54 viable units per animal.

The day after challenge half of each group was started on treatment with isoniazid, 10 mg per kg. of body weight per day, given by injection on six days of the week. At intervals during and after the period of treatment, pairs of animals from each of the four groups

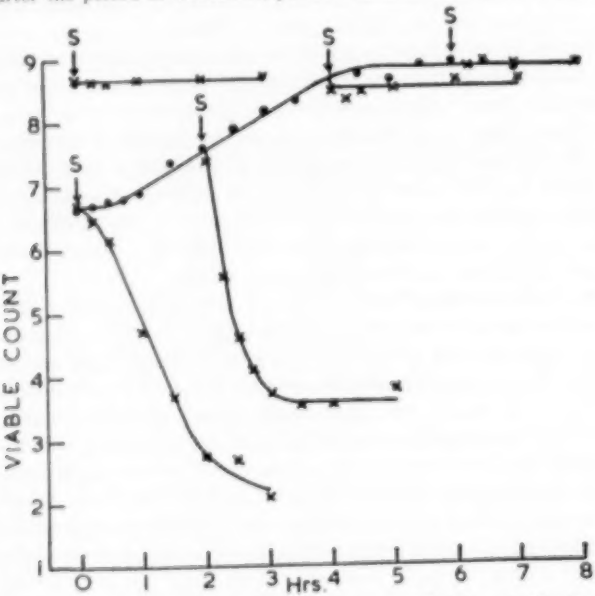


FIG. 1. The bactericidal activity of streptomycin on different growth phases of *Staph. aureus*.

—●—●— growth curve in absence of streptomycin.
—×—×— viable counts after addition of streptomycin, 50 γ per ml., at times indicated by *S*.

were sacrificed, half of the spleen was examined histologically, and a viable count was carried out on the other half after grinding with a Fluon® (Imperial Chemical Industries; equivalent of Teflon®) grinder (7). Guinea pigs not previously BCG vaccinated were tested at intervals for the development of allergy by intracutaneous injection of 0.1 ml. of a 1:100 dilution of Old Tuberculin.

## RESULTS

### *The Influence of the Growth Phase of Cultures on the Activity of Streptomycin and Isoniazid*

Nutrient broth cultures of *Staph. aureus* were grown for eighteen hours and were then diluted 1:100 in fresh broth. Samples from the eighteen-hour culture and at intervals from the diluted culture were put into bottles containing sufficient streptomycin to make a final concentration of 50 γ per ml. Viable counts were then done at intervals on the culture with and without streptomycin.

The results are shown in figure 1. It may be seen that streptomycin had a rapid bactericidal activity on samples taken during the lag phase and the early logarithmic phase of growth, but had little effect on samples taken at the end of the logarithmic phase. Streptomycin had no effect whatsoever on the eighteen-hour culture or on the six-hour culture in the stationary phase over a three-hour period.

Further experiments also have demonstrated that the inactivity of streptomycin on stationary phase organisms is not due to drug resistance, destruction, or inactivation of the streptomycin, and that a bactericidal effect is immediately obtained if the culture is merely diluted.

Similar experiments on Tween®-albumin medium cultures of *M. tuberculosis*

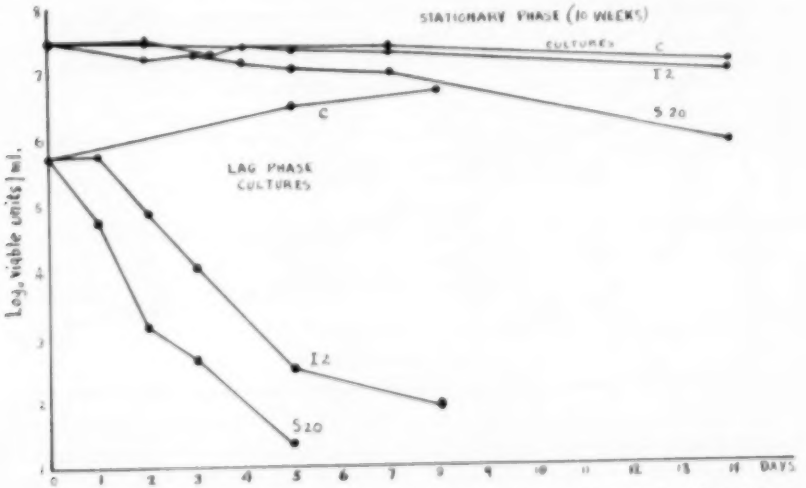


FIG. 2. The bactericidal activity of streptomycin and isoniazid on lag and stationary phase cultures of *M. tuberculosis*. C, control culture without drugs; I2, culture containing isoniazid 2 γ per ml.; S20 culture containing streptomycin 20 γ per ml.

are shown in figure 2. Here it may be seen that lag phase cultures are rapidly killed by streptomycin, 20 $\gamma$ per ml., or isoniazid, 2 $\gamma$ per ml.; whereas streptomycin only caused about a tenfold reduction in the viable count of a stationary phase culture over a fourteen-day period, and isoniazid had little or no effect during the same period.

*The Influence of Anaerobic Conditions on the Activity of Streptomycin and Isoniazid*

Cultures of *M. tuberculosis* were grown in Tween-albumin medium for ten days and were then diluted 1:10 in bottles of fresh medium to which was added streptomycin, 20 $\gamma$ per ml., or isoniazid, 2 $\gamma$ per ml. Some of these cultures were then incubated under aerobic conditions, and others under anaerobic conditions in a MacIntosh-Fildes jar. At intervals, viable counts were carried out, and the results are shown in figure 3. It may be seen that anaerobic conditions in the absence of added streptomycin or isoniazid completely inhibited growth over a ten-day period. Anaerobic conditions slowed to a considerable extent the bactericidal activity of streptomycin and abolished almost completely the bactericidal activity of isoniazid.

As a control, to ensure that the action of streptomycin was not inhibited by anaerobic conditions, cultures of *E. coli* were subjected to the same concentrations of streptomycin under aerobic and anaerobic conditions. No difference was

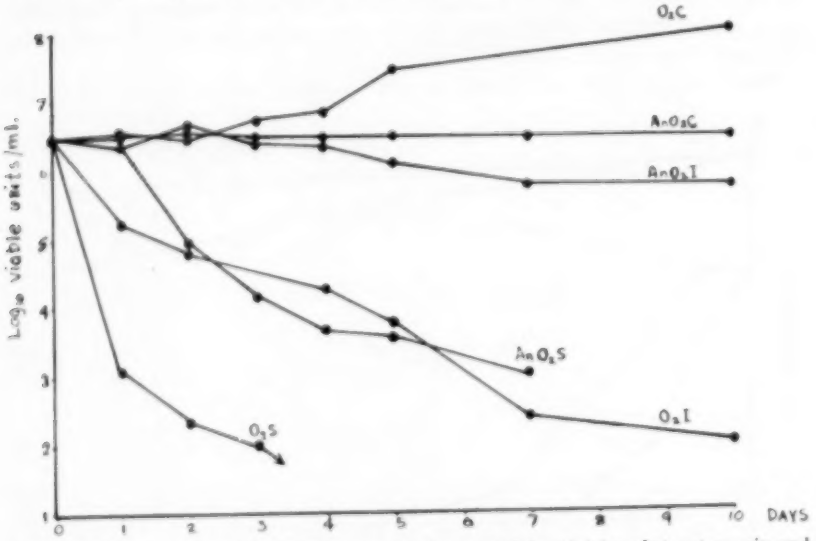


FIG. 3. Effect of anaerobic conditions on the bactericidal activities of streptomycin and isoniazid. $O_2C$, culture without drugs under aerobic conditions; $O_2S$, culture containing streptomycin, 20 $\gamma$ per ml., under aerobic conditions; $O_2I$, culture containing isoniazid, 2 $\gamma$ per ml., under aerobic conditions; $AnO_2S$, culture containing streptomycin, 20 $\gamma$ per ml. under anaerobic conditons; $AnO_2I$, culture containing isoniazid, 2 $\gamma$ per ml., under anaerobic conditions; $AnO_2C$, culture without drugs under anaerobic conditions.

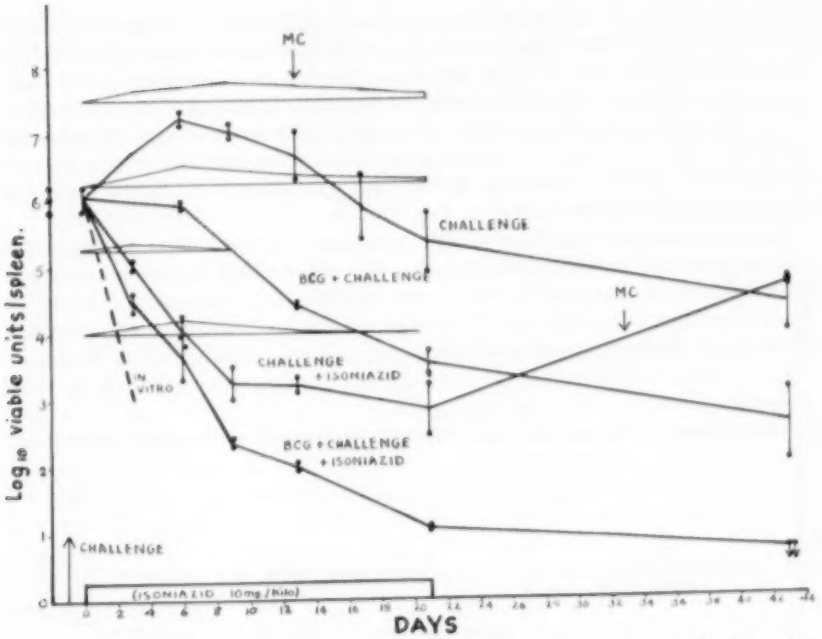


FIG. 4. The bactericidal activity of isoniazid in guinea pig spleens in animals unvaccinated or previously BCG vaccinated. Challenge infection with attenuated Branch strain of bovine origin. The continuous curves are the viable counts on the spleens of animals sacrificed at intervals after challenge. The dotted line represents the average of the *in vitro* bactericidal activity of isoniazid, 2 $\gamma$ per ml., on lag phase cultures. The triangular segments represent the degree of inflammatory response in the spleens, the height from the base line indicating in arbitrary units the extent of the response. These segments from above downward indicate the responses in the challenge, BCG + challenge, challenge + isoniazid and BCG, challenge and isoniazid groups, respectively. *MC* indicates the date on which skin allergy first appeared in the two unvaccinated groups.

found in the bactericidal activities of the drug in the two sets of circumstances. Moreover, there was no difference in the growth rates of cultures free of streptomycin either in the presence or absence of oxygen.

### *The Influence of Previous BCG Vaccination on the Bactericidal Activity of Isoniazid in Guinea Pigs*

The results of enumeration of the populations of tubercle bacilli in the spleens of guinea pigs challenged with the attenuated Branch strain of *M. tuberculosis* are shown in figure 4. In unvaccinated, untreated animals the viable counts rose during the first six days, and then steadily declined until forty-three days after challenge. In BCG-vaccinated, untreated animals there was no initial rise in the counts, and the subsequent fall was similar in rate to that in the unvaccinated animals.

The counts in unvaccinated, treated animals fell sharply for nine days at a rate only slightly lower than that found with lag phase cultures subjected to isoniazid, 2 $\gamma$ per ml., *in vitro*. However, between nine and twenty-one days there was little or no fall in the viable counts. This flattening of the curve could be attributed to both the presence of metabolically inactive organisms in the bacterial population and a decrease in the macrophage response of the defense mechanisms, as demonstrated by the degree of infiltration in the spleen, indicated diagrammatically in figure 4.

Isoniazid-susceptibility tests on the recovered organisms demonstrated that the majority of the bacterial population was still susceptible. After treatment was discontinued, the counts rose again, and Mantoux conversion occurred on the thirty-third day in contrast to the experience in the untreated group, in which it occurred on the thirteenth day. Thus, there is some evidence that treatment with isoniazid had prevented the development of immunity and allergy in these animals.

The counts on the guinea pig spleens in vaccinated and treated animals fell

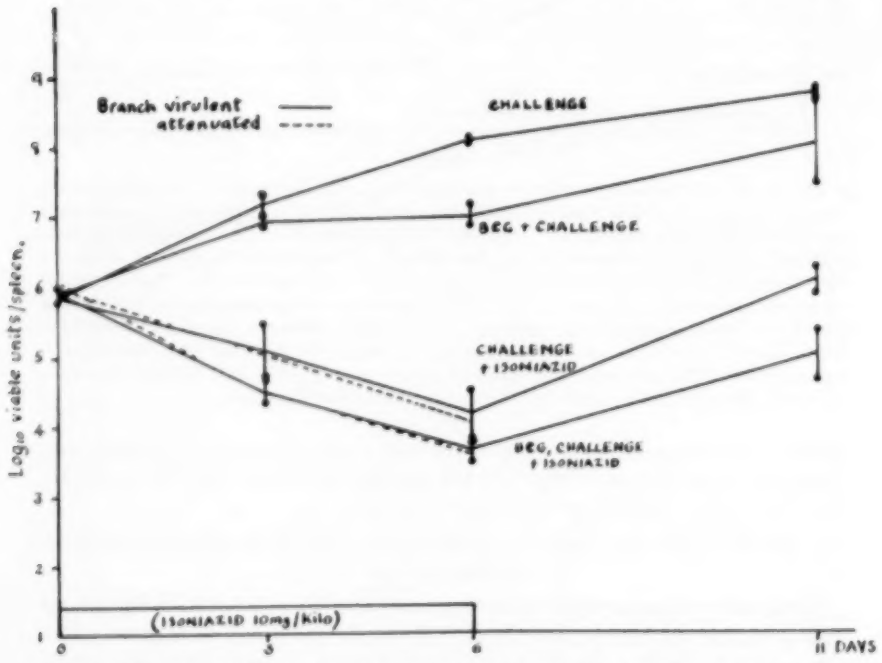


FIG. 5. The bactericidal activity in guinea pig spleens in animals unvaccinated or previously BCG vaccinated. Challenge infection with virulent Branch strain. The continuous curves are the viable counts on the spleens of animals sacrificed at intervals after challenge. The dotted line represents the relevant portion of the curves from a similar experiment with the attenuated Branch strain shown in figure 4.

more rapidly than in the unvaccinated group during the first nine days and, although the curve tended to flatten, it was still steeper from nine to twenty-one days. It seems probable that the continued fall in the viable counts during this latter period was mainly due to the macrophage response which persisted for a considerably longer period than it did in the unvaccinated, treated group.

## DISCUSSION

From the *in vitro* observations described, it seems probable that either the achievement of maximal population densities of tubercle bacilli or the presence of anaerobic conditions of growth may limit the bactericidal activities of streptomycin and isoniazid. Such conditions may occur in certain types of tuberculous lesions, such as the interior of caseous lesions. However, the effect of a heightened immunity reaction in the guinea pig spleen increased the apparent bactericidal activity of isoniazid.

It seems probable that in this situation the immune response is primarily bactericidal, as demonstrated by the marked fall in spleen counts in untreated animals infected with an attenuated strain which, nevertheless, could eventually be lethal. Thus, the situation is analogous to the synergism shown between any two bactericidal drugs by Jawetz, Gunnison, Bruff, and Coleman (8), and Singh and Mitchison (5, 9).

It could be argued that the total bactericidal activity demonstrated in the guinea pig spleens was due to the defense mechanisms alone. This is unlikely, since the fall in the viable counts was most rapid in the early stages in which there was little evidence of histologic response and when the counts were either rising or were stationary in the untreated groups.

Finally, it is most important to emphasize that the synergism demonstrated between the degree of immunity and the bactericidal activity of isoniazid has only been shown for one organ in one animal species with one particular type of defense mechanism. It would seem most unwise to generalize these findings to the treatment of human disease.

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## GENERAL DISCUSSION

Dr. Middlebrook: The papers by Drs. McDermott and Mitchison are now open for discussion.

Dr. Long: I want to address one question to both Dr. McDermott and Dr. Mitchison with regard to the apparent persistence of bacilli in the spleen, of bacilli in such forms that they can be detected some months later, but not at the time of first examination.

Do you know that these bacilli have been there all the time, or could they not have come from the lung which still contains plenty of bacilli? There is probably a very simple answer to this question.

Dr. McDermott: I forgot to state we did do one very large experiment, an experiment in which the bacilli vanished from the spleen and in which we also studied all the lymph node tissue we could get, plus kidney, plus bone marrow. In other words, all available tissue, and it came out the same way: no cultivatable bacilli.

Dr. Long: Lung, too?

Dr. McDermott: Oh, yes, the lung, too. The lung is actually done routinely, but we did all the other tissues we could find, and it still came out the same way. The possibility which you suggest, of course, is not entirely excluded.

Dr. Mitchison: May I try to answer that part of Dr. Long's question which was addressed to me? In guinea pigs the extent of the disease is always very much more marked in the spleen than in any other organ. Furthermore, we did examine the liver and lungs histologically, though we didn't do counts on these organs. Since the lesions in the liver and lungs were less numerous and also responded to chemotherapy, it would seem improbable that they were the source of the persisting bacilli found in the spleen.

Dr. McDermott: Could I make another point? Of course, it should be pointed out for the benefit of the audience that we are dealing with a sampling technique in that it isn't always the same mouse ninety days afterwards. Nevertheless, our conclusions with the sampling technique do seem justified.

Dr. Long: I understand that.

Dr. Kassowitz: I would like to speak on the subject of pyrazinamide in combination with other antituberculous drugs, not from the experimental angle, but from the standpoint of a very unusual case of meningitis that developed in a streptomycin-PAS-treated child after being successfully—apparently successfully—treated. After five or six months of treatment, meningitis developed.

We made cultures and resistance studies and, when they came back, we had started isoniazid which should have been given before, but it wasn't. Isoniazid did not seem to improve the condition. We found isoniazid resistance and streptomycin resistance, but PAS susceptibility.

Then pyrazinamide was given and PAS was given intravenously. The child made a complete recovery apparently on pyrazinamide in combination with PAS. I think this case may be pertinent to Dr. McDermott's remarks.

Dr. Davis: I would like to ask Dr. McDermott: when he gets the stabilization curve on any drug, and then, say, doubles the dose of that drug, what happens to the level of the ordinate?

Dr. McDermott: We haven't done that. We have used very large doses, however. The dose of isoniazid in per kilo terms, which is a crude measurement, was around 25 mg. per kg. per day.

As you know, if one gives a mouse the amount of streptomycin which will cure many a man of miliary tuberculosis, the mouse will go ahead and die of tuberculosis.

One has to "up" all the doses. We haven't studied dosage specifically but we are in the process of doing it now, particularly with smaller inocula. I would doubt very much if it had any effect. We feel pretty secure on the point.

DR. GLADYS HOBBY (Brooklyn, New York): I just wonder if that is really a large dose in terms of dosage of all the drugs we use. I wonder what others feel about that. We use so much larger doses for the mouse species than for the higher species of animals. I would consider that 25 mg. per kg. per day just about an average dose on the mouse. I would still be curious to know what happens on higher doses.

DR. MCDERMOTT: We also give 4 mg. of streptomycin along with it and give PAS along with it and still get the same result.

DR. MITCHISON: Have you done blood levels?

DR. MCDERMOTT: No.

DR. KOCH-WESER: We used an entirely different technique, but our results probably mean the same as Dr. Mitchison's. When one adds carbon$^{14}$ labeled isoniazid to tubercle bacilli in the logarithmic phase, they stop growing after about eight to ten days, and, as Dr. Middlebrook published, lose their acid-fastness entirely. This happens under entirely favorable metabolic conditions for the tubercle bacillus which then takes up and binds C$^{14}$isoniazid firmly. If C$^{14}$isoniazid is added under unfavorable conditions, in the icebox or after previous treatment for one-half hour with 45°C. temperature, the tubercle bacilli take up considerably more of the radioactive isoniazid than the actively metabolizing bacilli do.

Now, we explain this by physical absorption without metabolic utilization. The difference between the two uptakes is the following. The ones that took up isoniazid at 37°C. under favorable metabolic conditions, if they are resuspended in fresh medium, retain the radioactivity. They retain the C$^{14}$ isonaizid entirely, and nothing, or merely 5 per cent of it, is found in the medium after about ten days.

If they take it up under unfavorable conditions, they do not lose the acid-fastness in the first place. They remain acid fast throughout.

Second, resuspended in fresh medium, they lose their radioactivity within ten days. About 80 per cent of it is found in the medium, and only about 10 or 20 per cent is retained in the bacilli.

Third, they grow out from this medium. It seems that isoniazid is really metabolized and really becomes bactericidal only under favorable conditions; only if the tubercle bacilli metabolize well, as Dr. Mitchison so beautifully showed.

The last point is important: If one adds streptomycin to the cultures first, and lets streptomycin inhibit the growth of those bacilli for three or four days, and then one adds the radioactive isoniazid, the tubercle bacilli do not lose acid-fastness and do not fix isoniazid. In other words, the streptomycin has created an unfavorable metabolic condition.

There might be the same situation giving pyrazinamide first and then isoniazid. Is that possible?

DR. MCDERMOTT: Well, that gets very complicated. The reason I say that is we have done studies such as Dr. Mitchison's *in vitro* with pyrazinamide and have not been able to reproduce the phenomenon *in vitro*, the eradicative type of action, even when we lower the pH. I just don't think we are entitled to any speculations about what pyrazinimide does or does not do to tubercle bacilli *in vitro*.

I do think these observations of Dr. Koch-Weser's are terribly important with respect to the rest of the problem.

I would like to say for historical purposes because it isn't said often enough: this lady on my right, Dr. Hobby, originally showed this phenomenon in about 1940.

The basic phenomenon is that sulfonamide, for example, would stop penicillin action. All the rest of us, including the very beautiful studies of Dr. Mitchison and Dr. Koch-Weser *in vitro* on these questions, have really been elaborating on a basic phenomenon originally described by her.

DR. MIDDLEBROOK: I was certainly ignorant of that fact! Dr. Hobby worked with the system sulfonamide-penicillin in 1940?

DR. HOBBY: 1942.

DR. MIDDLEBROOK: Everybody should remember that because, personally, I think that this particular problem, whether we use the term "persisters," or the term "physiologic insusceptibility" is very important (Dr. McDermott likes the latter. Dr. Mitchison likes "persisters." I don't know what term you use, Gladys. What did you use?)

DR. HOBBY: I don't think we gave it a label, frankly.

DR. MIDDLEBROOK: Just described the phenomenon?

DR. HOBBY: At that point we were undecided what resistance was.

DR. MIDDLEBROOK: This is not a problem of genetic resistance, is it?

DR. HOBBY: At that particular time we were undecided what was resistance. It was still too new in our minds. We had no terminology.

DR. MIDDLEBROOK: In any case, I think that the phenomenon is of great importance at the clinical level, not only in tuberculosis, but in other infectious diseases.

There is a chapter in a book (*Bacterial and Mycotic Infections of Man*, Lippincott, ed. 2, 1952) published under the auspices of the Poliomyelitis Foundation, the editor of which is here (Dubos). The author of the chapter is here (McDermott), in which this whole problem is discussed, I think, in most intelligent and sophisticated fashion, but you won't find a single—you will find very few—microbiologists who have ever read that chapter, I am sorry to say, Walsh.

They have no notion whatsoever what you are talking about, when you speak of the physiologic insusceptibility of "resting" bacteria. They think they must be genetically resistant organisms. It is not only theoretically important, but it is practically important, important in the clinic. Today it has enormous importance in chemotherapy of tuberculosis in my opinion.

[DR. DUBOS: I make it a profession to try to find discrepancies in statements made by the speakers, and I considered it good sport to follow Dr. Mitchison's presentation because it is so tightly knit I had little hope of finding any incompatible statements. But I think I found one.

He began in his usual lucid manner by differentiating two possible mechanisms through which a drug might fail to act, one being that the tissue environment doesn't permit the drug to function; the other being what we are now discussing: the bacilli find themselves in an unreceptive physiologic state. He dismissed the first possibility as most unlikely to be of any significance.

Now, it seems to me that, as I listened to his paper, every fact that he presented seemed to be in favor of that first possibility that he had dismissed. He demonstrated for example, by striking experiments that, when bacilli are placed in an anaerobic environment, the drug fails to act on them.

Well, it seems to me that there is one pertinent aspect of the chemistry of inflammation that has been well demonstrated. It is that the inflammatory area is an anaerobic environment so that the very fact that inflammation does take place under many circumstances, and does create an anaerobic environment, would explain according to his own results *in vitro* that in many cases the drug cannot possibly function even though the bacilli be in a fairly young physiologic state and even though the drug can penetrate the lesion.

I hope Dr. Mitchison can reconcile that first statement of his with his subsequent experimental findings.

Now, I would like to say a few words concerning the techniques of experimentation used by Dr. McDermott because I believe that these techniques have been used most widely in our laboratory, and I have great confidence in their significance. But I also have become painfully aware, during the past few months, of some of their limitations. I shall mention only two of these limitations.

Suppose that the number of bacilli present in the tissues is small—that those numbers

are small enough, that you cannot afford to dilute the tissue which you use for inoculating the culture medium.

If you are in that situation, I believe it is most unlikely that you will be capable of demonstrating through these techniques the presence of a small number of bacilli even though they be present in a living form because there is absolutely no doubt that all tissues do contain or do release, in the process of grinding—that is inherent in the technique—substances that are inhibitory to the multiplication of a small number of bacilli.

Those inhibitory substances are not too abundant in the spleen. That's correct. So, in general, the counts in the spleen from my experience are fairly convincing, and failure to find bacilli has some weight.

However, if you search for bacilli in the liver, you will find that, before you can cultivate them, you have to have an enormous number of living organisms in the liver to obtain positive cultures. You can demonstrate that very readily by taking normal liver, grinding it, and adding a known number of bacilli. They fail to grow.

In this respect, the lung occupies a position half-way between spleen and liver. Moreover, we have found that diseased tissue, diseased lung, is much more likely than normal lung to contain inhibitory substances. Thus, here is one very serious limitation to the technique that Dr. McDermott and we use.

Dr. McDermott recognizes that limitation, and introduced the statement to the effect that they had added a small number of bacilli, as he said it, from a "store-bought" culture, to his spleen suspension, and found that they grew; and that appears on first sight to be a valid control. I assume that control culture was made in spleen emulsion.

DR. MCDERMOTT: Yes, and lung emulsion.

DR. DUBOS: It would not apply to the liver, I can assure you. But it is not a complete control because, as you mentioned, they were "store-bought" bacilli, and "store-bought" bacilli are usually pretty young cultures, in our experience. This is an experience which has been wonderfully documented recently at the International Children's Center in Paris. As soon as the culture gets a bit older, as soon as you use a culture two or three weeks old—instead of one week old, as you probably use—you will find that it becomes so much less able to grow in ordinary culture media that it will even completely fail to multiply under these conditions. Since you are very likely dealing with precisely that kind of aged culture, weakened, having lost some of its physiological potentialities, I think the adequate control has not yet been worked out. I do not believe that any of the negative results obtained by culture technique should be taken as meaning that there were no live bacilli in the organs.

DR. MIDDLEBROOK: Dr. Mitchison first.

DR. MITCHISON: There were two points that Dr. Dubos brought up.

The first point was the question of the effect of anaerobic conditions.

Now, it is conceivably possible that the activity of streptomycin might in some way be peculiarly prevented or inhibited under anaerobic conditions, unrelated to the metabolic state of the bacilli. However, we have carried out a controlled experiment on this question. That is, we used a strain of *E. coli* which, of course, grows perfectly well under anaerobic conditions. Under these conditions the bactericidal activity of streptomycin is entirely unaffected.

DR. DUBOS: That is not a valid reason because *E. coli* does grow anaerobically. So it does not answer the argument. It does not answer my objection. *E. coli* can grow just as well anaerobically as aerobically, but the tubercle bacillus does not.

DR. MITCHISON: I do not think it is possible so to analyze the situation with tubercle bacilli that you can definitely distinguish between the possibility of inactivation of the streptomycin and the possibility of metabolic inactivity of the bacilli.

I think, nevertheless, that, if anaerobic conditions played a predominant role in the immune response in the animal, you would expect to see some degree of antagonism between the high degree of immunity and the activity of a drug such as isoniazid. On the

second point that Dr. Dubos raised, in connection with technique: you can improve the situation very greatly by using the deep cultures which we used in these techniques. You can dilute your spleen suspension to a far greater extent than you can on surface culture, and in this way you can get your dilution of organ suspension to a level where it almost certainly is not inhibitory.

Dr. Middlebrook: With regard to the first question that Dr. Dubos asked Dr. Mitchison—about the lesion—I would like to ask Dr. Mitchison whether the lesions he observed in the animals that were vaccinated and treated with isoniazid manifested any evidence of necrosis, any level of inflammation sufficient to lead you to believe there could be significant anaerobiosis in those lesions.

Dr. Mitchison: Certainly, in both groups, in the immune and the nonimmune animals, there were significant focal accumulations of histiocytes and macrophages, and there was early necrosis.

I wouldn't say there was marked necrosis, but there was early central necrosis.

Dr. McDermott: Taking that up first, I would like just to say that Dr. Dubos and I, of course, have had many discussions on this point, and our group is in complete agreement with him. When we were in the lamentable position of not finding any tubercle bacilli by any means in these animals, then these points were a great problem to us. But, once we got into the position where we could demonstrate the bacilli in at least a third of the animals ninety days later, it made no difference because it was obvious they were there. And the important thing was they vanished, which word was chosen very precisely. They had vanished under certain specific conditions and did not vanish in any other comparable situation.

I might say that one minor control which still isn't a complete control—but which might come to your mind—is that we have treated the animals, uninfected animals, with large doses of these various drugs, then ground up the spleens to which we added "store-bought" bacilli, and the results were the same.

Now, I would like to make some general remarks which have to do with Dr. Mitchison's paper, with Dr. Koch-Weser's work, with some of my own answers, and the rest of it on this whole subject. My remarks constitute a plea and the plea is as follows: Nobody knows that the populations of infecting parasites within the body are so strikingly regimented as they are in the conditions which operate on the left-hand side of anybody's curves at the beginning of an experiment. In other words, despite all of these things we have been talking about—no matter how much the line may slant down—we cannot eradicate tubercle bacilli from a culture: if one looks at the usual curve, it is evident that the population decline doesn't go all the way down to the bottom. It stops somewhere.

Many of these observations have to do with phenomena which might only apply within the body to one bacillus every Tuesday—something unimportant in so far as the total antimicrobial effect upon that infection is concerned. In other words, what I am saying is: There must be—or it is at least reasonable to assume that there must be—considerably more physiologic heterogeneity *in vivo* than *in vitro*. There are more bugs and more different kinds of states within the human body than is ever the case in our test tubes or animal experiments. Our experiments may apply only to the occasional situation in the occasional lesion.

Dr. Ström: I would like to address a question to Dr. Koch-Weser with regard to what he said about the isoniazid incorporated, as I understood it, in tubercle bacilli.

That is more or less a question of a technical nature, but it is quite important to understand the mechanism of action of drugs on bacteria. First, you said that after a certain time—and what time was it?—you find that 80 per cent of the isoniazid goes back again to the medium. I would like to know how you know that it was isoniazid.

I mean that labeling the tubercle bacilli may start a chain labeling of other metabolites, so the radioactivity you measure in either case may not be radioactive isoniazid. After a certain time, if you have the isoniazid incorporated in the bacilli, are the bacilli stable enough to be unchanged and then deliver the isoniazid back to the medium?

DR. KOCH-WESER: The second question I didn't understand. But, with regard to your first question: This was one of the main difficulties in the whole study which our group, Dr. Ebert and Dr. Barclay, has met. We never actually knew exactly, for instance, in those studies on distribution of radioactive isoniazid in tuberculous lesions, whether the substance we measured as radioactive was really isoniazid. However, in this particular instance, we have some indication that it is isoniazid in the simple fact that at least the substance re-secreted into the medium behaves like isoniazid as an antimicrobial agent.

DR. STRÖM: How long a time does it take for the turnover of the isoniazid again to the medium?

DR. KOCH-WESER: It goes stepwise: eighty per cent ten to twelve days after resuspension into fresh medium. After two days—the first time we tested the material—there was already about 10 per cent, a significant amount, in the medium.

The radioactivity was slowly coming out from the bacilli into the medium, together with the beginning of the growth curve.

DR. STRÖM: The next question—I will simplify it: don't you think that, when you have the isoniazid really incorporated in the bacilli, it kills the bacilli?

DR. KOCH-WESER: Yes, this is one of the answers Dr. McDermott gave. We found that all of our bacilli became nonacid-fast, in the opinion of several people doing really very intense research on slides and a great number of slides; we were not able to find any acid-fast bacilli in our cultures. Actually, tubercle bacilli started to disintegrate in that instance. We were never able to wash them, to get growth in fresh medium, if they were nonacid-fast.

Dr. Middlebrook pointed out in early papers on this subject that it unfortunately makes research more difficult. In a large number of cultures originally isoniazid-resistant bacteria are present. So that at the end of ten days or so, when only 90 per cent are nonacid-fast, if one resuspends those and they grow, the bacilli growing out are isoniazid resistant.

DR. MIDDLEBROOK: I didn't mean to imply that in anything that I have written. As a matter of fact, I have never yet seen a smear, and I don't know whether anybody in our laboratory has observed a culture of tubercle bacilli exposed to isoniazid, for I don't know how long, which doesn't still contain some acid-fast rods which are not drug-resistant mutants. Am I wrong, Dr. Schaefer? Have you seen any?

DR. W. B. SCHAEFER (Denver, Colorado): No. A few acid-fast rods always persist.

DR. MIDDLEBROOK: One per cent, 10 per cent? You can always find them. Are you talking about something so esoteric as trying to look for three hours, Dr. Koch-Weser? You would not have to look so long.

DR. MCDERMOTT: You say it doesn't happen with uniformity. Occasionally you get them all.

DR. KOCH-WESER: That's right. It depends largely on the time at which you add the isoniazid. At the sixth day, the beginning of the logarithmic phase, it works very well. During the later stage of the logarithmic phase, it fails. The oxygen supply is not adequate then. You do not get complete nonacid-fastness.

DR. MIDDLEBROOK: With regard to the experimental facts in general, when you get 95 per cent of the bacilli losing their acid-fastness, it is not just 95 or 99 per cent loss of viability. It is 99.5 or higher so that many of those that are still acid fast must still be unable to take off, to start multiplying again in the new environment.

DR. RIST: I should like to reassure the audience about the experiment of Koch-Weser and Barclay which should make us anxious about the action of streptomycin plus isoniazid together.

Dr. Koch-Weser stated that, if you put streptomycin and isoniazid together, then the bacilli don't take up the isoniazid and don't lose their acid-fastness. It is quite true. In 1952 I described the phenomenon. But even when the bacilli don't lose their acid-fastness in a mixture of streptomycin and isoniazid, the curious thing is that the bactericidal activity of the mixture is even greater than when there is only streptomycin or isoniazid in the medium. The inhibition of loss of acid-fastness doesn't at all inhibit the sterilizing

effect of the mixture. That proves that the losing of acid-fastness is not necessary for the killing of bacilli.

DR. MIDDLEBROOK: Would your observations permit you to add anything to that, Dr. Mitchison?

DR. MITCHISON: I don't think so, really.

DR. MIDDLEBROOK: Essentially the same experiences?

DR. MITCHISON: Yes, I should think so. I thought the main point was that streptomycin is presumably a more active bactericidal agent under most experimental conditions and might get a chance of killing the bacilli before the isoniazid does. Therefore, perhaps, the morphologic changes that occurred in the majority of the bacilli were due to streptomycin-killing rather than to isoniazid-killing.

DR. HOBBY: In this connection, I should like to describe a series of very simple experiments which we have recently completed. Taking tubercle bacilli, susceptible to isoniazid and susceptible to streptomycin, running an ordinary growth susceptibility test to streptomycin in a liquid medium, we have then selected those tubes in which there was a concentration of streptomycin that was not sufficient to inhibit growth. In other words, if our end point for a susceptibility was about 0.6 mg. streptomycin per ml., we selected a tube containing about 0.3 mg. streptomycin per ml., for example. We have then used the organisms from such a tube to determine the effect on isoniazid, using routine growth curves, much like those that have been described today. So far we have had no exceptions to the rule that those organisms have then been apparently physiologically resistant to isoniazid. On the other hand, when we have worked with streptomycin and isoniazid in combination—mixtures of the two—there has been no indication of streptomycin's interfering with isoniazid. I have rather had the impression that probably the isoniazid got there first and streptomycin was just "along for the ride."

DR. KOCH-WESER: This is true. Streptomycin has to be added first for about two or three days, at least. We also did the experiment, as you point out, adding both drugs at the same time. Then the effect does not appear. Isoniazid probably gets there first. The bacilli have first to be rendered inactive by streptomycin before the isoniazid gets in touch with them.

DR. STRÖM: The same time, the loss of acid-fastness doesn't occur.

DR. KOCH-WESER: Occurs in part.

DR. RIST: The two things are equivalent.

* * *

[Subsequent to this discussion, the Editor became aware of the paper by Dr. Lack bearing on this subject. Dr. Lack prepared the following comments for inclusion in the record at this point.

DR. LACK: In my study of "The Significance of Pleomorphism of *M. Tuberculosis* var. *Hominis*" (J. Gen. Microbiol., 1953, *8*, 18), I reported that under a variety of adverse conditions the tubercle bacillus may continue to divide but more slowly and in smaller forms. The reduction in length may be from 7 $\mu$ to 2 or 3 $\mu$. I found no evidence of an *L* cycle or of what are considered to be filter-passing forms, but these short rods induced by starvation, by reduced oxygen, by streptomycin, and by isoniazid are modified bacteriologically as well as morphologically. They do not grow in a corded pattern; moreover, those obtained by culturing in sublethal concentrations of streptomycin are more resistant to isoniazid.

We find similar short forms in chronic skeletal tuberculous foci and in caseum from chronic lung lesions. These are difficult to culture, but are capable of growing back to normal bacilli and of producing typical tuberculosis in guinea pigs when the biopsy material is properly handled. When we implant caseous material, containing these short forms, directly into a muscle pouch in the guinea pig's thigh, we have a significantly higher percentage of positive pigs than when we grind up this material and inject the suspension. The slow digestion of the caseous material in the muscle pouch, which may take a week or two, appears to favor the survival and growth of these dormant bacilli; this may be be-

cause we are reproducing a softening more comparable to environmental changes to which bacilli in caseous foci are subjected in nature.

I have mentioned that I found no evidence of an *L* cycle in studying slide cultures of tubercle bacilli under a wide variety of conditions. Nevertheless I would like to introduce the concept that regression to a smaller and less complex form may be a mechanism by which some bacteria, including tubercle bacilli, adapt themselves to an unfavorable environment. I term this change "regression" for the following reason. An organism in the course of its evolution has progressed from simple to more complex structure; natural selection among parasites has favored mutants better equipped to withstand host defense mechanisms. The possession of a capsule by a pneumococcus or of so-called "cord factor" by the tubercle bacillus may be important for survival under certain conditions, but under other conditions it may be equally important for their survival that these organisms should be able to grow without having to synthesize these substances. Furthermore, there is some evidence for the hypothesis that regression to less metabolically active forms, whether by sporing or by going into the *L* phase or by becoming "small forms" or *G* forms, may not only ensure survival under certain adverse conditions but may allow subsequent development, progression, along a different metabolic pathway better adapted to local conditions. (LACK, C. H.: Adaptation in micro-organisms. Third Symposium of the Society for General Microbiology. London, April 1953, ed. by E. F. Gale & R. Davies. Cambridge University Press, 1953, p. 251.)]

# COMPLICATIONS OF SKELETAL TUBERCULOSIS AND ITS TREATMENT

CHARLES H. LACK[1]

When asked to talk about skeletal tuberculosis at this Symposium, I was faced with a dilemma. There has been such a decline in this disease since the war, and antimicrobial therapy has allowed earlier surgery, accelerated healing, and has prevented complications to such an extent that the disease today is very different from the disease at the time when it was possible to study larger numbers of cases.

I am, therefore, restricting myself to a few complications which surgeons perhaps rather take for granted, but which are intriguing in their implications to the pathologist.

These complications are set forth below because, otherwise, the topics I mention might appear unrelated.

Complications of Tuberculous Infection:
- (*a*) Trauma, which may be followed by frank disease
- (*b*) Cortisone and corticotropin, which accelerate pathogenesis

Complications of Tuberculous Disease:
- (*a*) Local sepsis: amyloidosis
- (*b*) Local extension of bony lesion to joint and adjacent tissues
- (*c*) Spread to other bones, kidneys, meninges, and lungs

Complications of Treatment:
- (*a*) Immobilization osteoporosis: flat chest, fractures, calculi, arrested growth
- (*b*) Drug reactions

*Complications of tuberculous infection:* Many surveys of skeletal tuberculosis mention trauma as an etiologic factor, e.g., Dobson (1) found that, among 914 patients with spinal disease, 131 gave a reliable history of antecedent injury. Petrov and others have shown that, if the limbs of animals are injured immediately before intravenous injection of tubercle bacilli, the traumatized area will become the site of disease in a high proportion of cases, and it has been assumed that a *locus minoris resistentiae* becomes infected by blood-borne bacilli. Sometimes, however, the clinical picture suggests that trauma, mechanical or chemical, has led to the proliferation of bacilli already dormant in the tissues involved; and the following cases are shown to illustrate this hypothesis.

The first patient to be cited is a boy who had a primary infection at the age of three and one-half years. Six months later, when he was well and gaining weight, he injured his right elbow. The roentgenogram taken two days after his injury (figure 1) showed neither fracture nor disease, but a few weeks later the boy developed a subperiosteal abscess and an active focus in his ulna (figure 2). He has not developed any lesions elsewhere.

The next patient is a boy of ten years whose fifth metatarsal styloid was fractured by a crush injury, and who developed a tuberculous abscess at that site, although he remained well (figure 3).

[1] Institute of Orthopedics, Royal National Orthopedic Hospital, London W.1, England.

FIG. 1. Roentgenogram of elbow of four-year-old boy two days after injury.

FIG. 2. Roentgenogram of elbow shown in figure 1, obtained two months later, showing rupture of tuberculous focus into joint, and a subperiosteal abscess.

FIG. 3. Roentgenogram showing tuberculous abscess at site of fracture of left fifth metatarsal styloid in a boy of ten years.

FIG. 4. Roentgenogram of pelvis of a boy of eighteen years who developed tuberculous abscesses on either side of his symphysis pubis following hyperabduction.

FIG. 5. Roentgenogram of pelvis of a boy with Still's disease at time of receiving corticotropin.

FIG. 6. Roentgenogram of pelvis shown in figure 5, obtained eighteen months later, showing gross destruction of hip joint and extreme osteoporosis.

The next roentgenogram shows the pelvis of a boy aged eighteen years who injured himself doing "splits" in the gymnasium. He developed tuberculous abscesses on both sides of his symphysis pubis (figure 4). A roentgenogram of his chest showed no abnormality, and he did not develop lesions elsewhere.

A middle-aged man struck his thumb while hanging up a picture. He did not break the skin or fracture the bone, and was otherwise fit, but developed a tuberculous dactylitis a few weeks later. Where do the bacilli come from in this type of case? I find it difficult to believe that there has always been a post-traumatic hematogenous spread.

A survey of 500 patients with skeletal tuberculosis in our hospital (2) showed that only 57 per cent had active pulmonary lesions and, although in children the skeletal lesion usually follows closely on the primary infection, in adults the interval between active pulmonary disease and skeletal or renal involvement may extend to decades.

The action of corticotropin and of cortisone is illustrated by two cases.

The first roentgenogram (figure 5) is of the pelvis of a boy of seven years who had Still's disease and was given an eight-day course of corticotropin. He developed a tuberculous destruction of his hip joint, illustrated in figure 6, which appeared to be unusually rapid. There was no activation of any lesion elsewhere. The diagnosis of Still's disease is not in doubt.

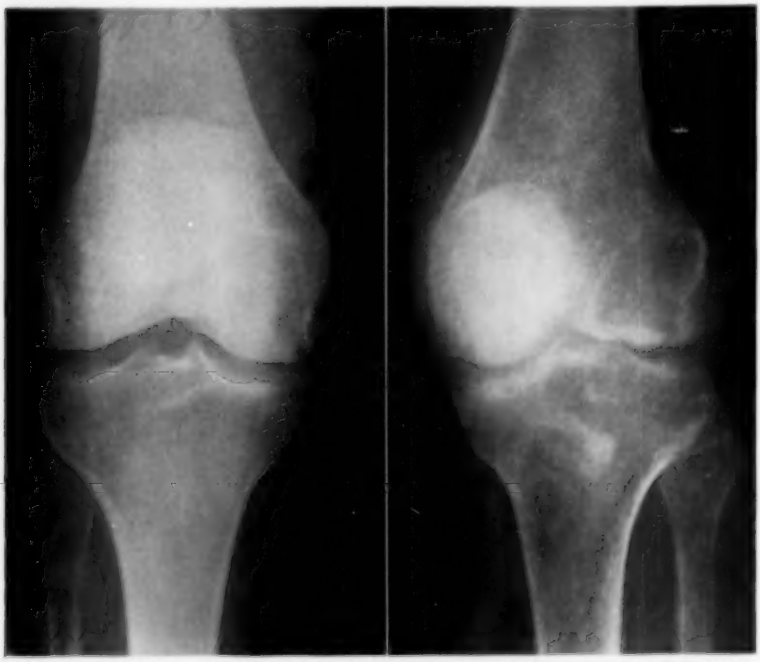

FIG. 7. Roentgenogram of knee joint of woman of fifty-three years with "arthritis" of eighteen years' duration.

(Left) At the time of receiving intra-articular cortisone.

(Right) One month later.

Figure 7 is a roentgenogram of the knee joint of a woman of fifty-three years who had had a "rheumatic knee" for eighteen years. The picture on the left shows her knee joint at the time of receiving intra-articular cortisone. She was given four injections of 50 mg. each. This was followed by a rapid tuberculous erosion, shown on the right. Investigations failed to reveal any abnormalities in her lungs or elsewhere.

I believe that hematogenous disseminations may occur shortly after the primary infection, but that the local environment may account for the long period of latency in the skeleton and in the kidney, and that mechanical and chemical trauma to those tissues may alter the milieu to produce manifest disease.

*Complications of tuberculous disease:* Secondary infection of skeletal tuberculous lesions is regarded as a serious complication. "To open an abscess is to open a door by which too often death will enter."

In sacroiliac tuberculosis, for instance, a sinus raises the death rate from 10 to 25 per cent during the critical first six years of the disease (3). Most of these secondary infections are staphylococcal, but it is not clear why the prognosis should be so gravely worsened.

Local extension of the tuberculous disease is not conspicuous—not by comparison with the enhancing effect of hyaluronidase—producing staphylococci on tuberculosis in guinea pigs, as reported by Bergqvist and Packalén (4). But extension can occur in dramatic form.

I recall the abrupt onset of dysphagia followed by rupture of an abscess into the esophagus of a patient with a tuberculous lesion of a thoracic vertebra which had somehow been invaded by staphylococci; and I also remember a child whose mass of caseous glands underwent abrupt liquefaction following a staphylococcal septicemia, although in her case the staphylococci were not found in the abscess.

Staphylococci and streptococci or their respective kinases may bring about the softening of caseum and the dissolution of fibrin, provided that plasminogen is present, although inhibitors in the form of long-chain polysaccharides or fatty acids may prevent proteolysis.

I might mention here that, in one respect, surgeons have almost welcomed the presence of staphylococci in a tuberculous joint, that is, to ensure bony union. Tuberculous granulation tissue may undermine cartilage but does not dissolve it, and fibrous ankylosis is to be expected. Staphylococcal pus will bring about rapid chondrolysis; the dissolution of intervening cartilage favors bony union.

I must omit any consideration of amyloidosis and of the other complications of frank disease, except to mention renal tuberculosis.

It is probable that the kidneys are infected by hematogenous spread at the same time as the skeleton, and early lesions are cortical. In a large proportion of patients these give rise to a transient intermittent and symptomless bacilluria. Most of these cortical lesions heal and are sealed off without macroscopic trace except for a few spots of calcification. It is only when foci in the cortex or pyramids ulcerate into the calyces that the condition is recognizable clinically. In the majority of our patients the renal lesion is discovered after the skeletal lesion, sometimes as long as fifteen years later.

The chronicity of this disease may be exemplified by one patient who has been

FIG. 8. Roentgenogram of knees three months after immobilization. The left knee is tuberculous.

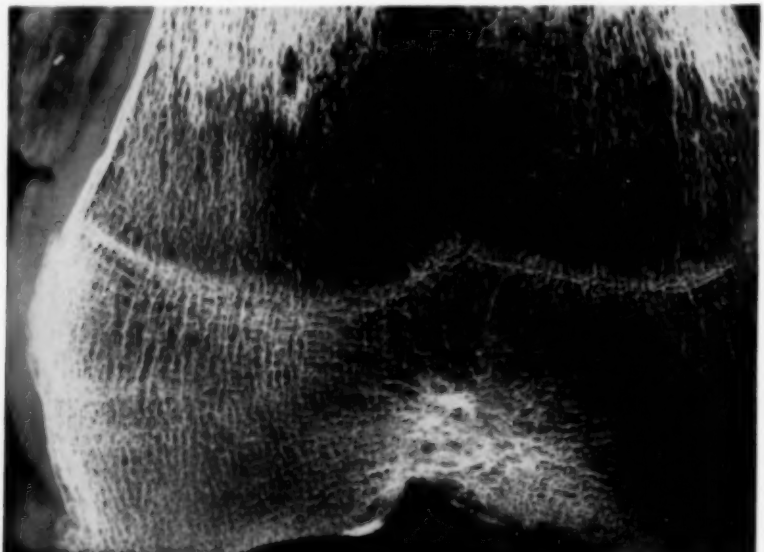

FIG. 9. Roentgenogram of an undecalcified slice of lower end of the left femur shown in figure 8.

FIG. 10. Roentgenogram of pelvis of child three and one-half years old with tuberculous left hip at diagnosis.

FIG. 11. Roentgenogram of pelvis shown in figure 10, obtained seven months after immobilization and one month after streptomycin therapy was begun. Note disappearance of femoral head.

excreting tubercle bacilli intermittently for more than forty years, although he looks and feels well (6). Renal involvement is not a particularly lethal complication of skeletal tuberculosis. It is found in 15 to 30 per cent of cases and it is much more frequent when there are multiple bone lesions.

*Complications of treatment:* The most important complication of treatment is osteoporosis. If you will look at the roentgenogram of a tuberculous knee joint (figure 8) three months after immobilization, you will see a diffuse porosis of the affected limb, with some areas of pronounced rarefaction. The left hip and the left foot, although not diseased, also show marked rarefaction after only three months in plaster.

Now let us look at the roentgenogram of an undecalcified slice of the lower end of the femur of the affected knee. You will see that there are three zones of bone loss in a tuberculous limb. First, at the site of infection granulation tissue replaces bone. This area is usually limited to a few millimeters from the bone surface in a joint infection. Beyond this is an area of porosis which increases as the disease progresses and may result in roentgenographic disappearance of the part. This corresponds to the highly porotic zone in the slab roentgenogram (figure 9).

The extreme osteoporosis of this zone appears to be related to the inflammatory reaction produced by the infection, and I think it probable that it reflects the degree of local blood stasis. As the infection is brought under control, this bone reappears roentgenographically.

FIG. 12. Roentgenogram of pelvis shown in figures 10 and 11, obtained two and one-half years later, showing return of femoral head.

The roentgenograms of the hips of a boy of three years illustrate this. The first (figure 10) was taken on admission and shows involvement of both joint surfaces of the hip and a sequestrum. After six months of conservative treatment, there is roentgenographic disappearance of the femoral head, shown in the next film (figure 11). The patient then received a course of streptomycin. The last film, two and one-half years later, shows the reappearance of the head (figure 12). Histologic studies show that this "infiltration" and bone loss is not tuberculous. It is comparable to the porosis which rapidly develops in the distal fragment of a fracture, and recovery from this form of bone loss occurs even during immobilization.

You will have noticed that in these roentgenograms there is a porosis extending beyond the inflammatory area, which is the result of immobilization; it will occur when a normal limb is immobilized. This general porosis, due apparently to the absence of normal stresses and strains on bone, may have serious consequences to the child immobilized for a long period.

Deformities may arise from the shearing away of porotic bones, especially vertebrae, from premature fusion of epiphyses, from flattening of the chest, and from fractures. The increased excretion of calcium may cause renal calculi. This porosis may persist for many years after activity is renewed, as shown in the last photograph, showing the pelvis and femora of a boy of eleven (figure 12). It is five years since he was immobilized.

The question which I would like particularly to throw into the discussion is whether bed rest or immobilization is really necessary in the treatment of skeletal tuberculosis when antimicrobials are available, in view of the serious consequences of immobilization porosis.

*Acknowledgment*

The writer wishes to acknowledge the help given by Dr. H. A. Sissons and Dr. F. H. Stevenson, who have allowed him to use their photographs.

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## GENERAL DISCUSSION

DR. MCDERMOTT: I just want to sharpen Dr. Lack's question after his admirable presentation. Are you questioning whether the rest is necessary for the treatment of the tuberculosis, or whether the rest is necessary in connection with the surgical therapy?

DR. LACK (London, England): I am questioning whether rest is necessary in the treatment of the tuberculosis itself.

DR. MCDERMOTT: If we have a plebiscite, I would say no, in this situation.

DR. MIDDLEBROOK: So would I, Walsh.

DR. MCDERMOTT: That's two votes.

DR. ATHA THOMAS (Denver, Colorado): This paper is very interesting to me as an orthopedic surgeon. There is one complication of immobilization which Dr. Lack mentioned but did not emphasize and which we have found very serious, although not common. That is the effect on the epiphysis of areas distal to the lesion whereby we get premature ossification. Not only do we get extreme shortening, but we get angular deformities from irregular ossification and fusion of the epiphysis, which result in very serious crippling. We have seen several of these, in which the deformity from the epiphyseal disturbance was really more crippling than the ultimate lesion from the tuberculous condition. It is most commonly seen in tuberculosis of the hip, when we get disturbances in the lower femoral epiphysis or the upper tibial epiphysis, with knock-knee or bow-leg and severe shortening.

At this hospital in the last year we have set up a special protocol for the handling of our skeletal lesions. We haven't had the nerve to apply this to the lower extremity yet, but in the upper extremity lesions which we see early, we have no immobilization whatsoever. They are treated entirely by chemotherapy. With the lower extremity and the spine, where you have the deforming influences of weight-bearing and muscle spasm, we hesitate to give them complete freedom of physical activity. We don't know that we cannot. If we can get these lesions early and can relieve the pain and retard the activity, possibly the muscle spasm can be eliminated and we will not get the deforming influences.

I do agree with Dr. Lack very emphatically that the complications of immobilization are serious and, as I say, may be even more crippling than the tuberculous lesion itself.

DR. EDITH LINCOLN (New York, New York): I just want to say that from a follow-up of children with primary tuberculosis, who developed bone deformity as a late complication, I quite agree with Dr. Lack that it is very difficult to concede that this is due to a fresh hematogenous invasion. I think in a follow-up of children who have been treated with antimicrobial therapy we will get more evidence on this problem.

Recently we have seen a child who had very severe miliary tuberculosis, who was treated with six months of streptomycin, a year of isoniazid, and three years of cortisone; and two months after the therapy was stopped the child began to limp, without any trauma that was evident, and obviously it had tuberculosis of the knee.

DR. KASSOWITZ: I would like to plead again that we draw the line. We cannot entirely abolish immobilization of weight-bearing bone lesions: we try to limit it as much as possible in Pott's disease.

After intensive antimicrobial therapy, followed by early orthopedic arthrodesis after a brief period, it has been possible for a long time under antimicrobial therapy to get them up on their feet. We now have seven patients (which isn't very many) who had operations in 1949, 1950, and 1951, and all of those children became taller than their predicted average height.

I want to say that early arthrodesis and spinal fusion does not inhibit growth, but makes it possible to put those children on their feet early, prevents osteoporosis, and does not inhibit growth and height.

DR. SIFONTES (Puerto Rico): During the past three years—during the isoniazid era—I have had the opportunity to treat thirty children with osteotuberculosis. This includes practically all the bones in the body. Most of these children have had active primary

tuberculosis, and the others had healed pulmonary lesions as evidenced by pulmonary calcifications.

In regard to the therapy, we have used isoniazid and streptomycin versus isoniazid and PAS, and we find no difference in the response to therapy.

In regard to complications, we have observed very little osteoporosis. We have not observed any meningitis in these patients, and I would like to point out that we have never been able to keep a child with osseous tuberculosis in bed. We just don't have enough personnel to keep the child in bed.

The first time I saw a case of this kind the child was in a mango tree eating mangoes, and I was shocked. Nothing catastrophic happened. The children got better. They had no osteoporosis. Maybe that will shed some light on this subject.

DR. WARING: I would like to ask a question. Dr. Hugh Burke, of Canada, has referred to the frequency of tuberculous pleurisy with effusion preceding Pott's disease. I would like to ask Dr. Lack if in his experience he has made this observation.

DR. LACK: This has occurred quite frequently in our hospital, and has been reported by Dr. Stevenson in the *Journal of Bone and Joint Surgery*, but I have not made any personal study of it.

DR. ROBINSON: The early part of Dr. Lack's paper, in which he described the onset of bone tuberculosis following the use of cortisone in several cases, brings up a very important practical question.

The steroid drugs are being used more and more in children who don't have tuberculosis—at least who we don't know have tuberculosis—and I would like to ask the group whether they deem it advisable to suggest that every child to whom steroids are about to be administered should have a tuberculin test done first.

Second, if that tuberculin test is positive, and if there is a good indication for the use of steroids, should isoniazid perhaps be given also?

DR. MIDDLEBROOK: I think we should have it recorded that this is one of the sort of answers to some of the problems that Dr. Dubos posed. If he would like to have a discussion of it before he leaves, it is perfectly all right with me.

DR. LONG: What I want to say is along the same line. I think it can be answered as part of the answer to Dr. Robinson's question.

What happened following the administration of cortisone was so striking that it raised this question in my mind: Is it possible, for example, that what we call the spontaneous development of a tuberculous lesion in bone might represent the lowering of resistance during a period of natural hypercortisonemia?

DR. STEFAN GRZYBOWSKI (Toronto, Canada): I would like to take up the point mentioned by Dr. Lincoln—this question of skeletal tuberculosis. This occurs in the majority of cases in hematogenous dissemination within a few months of primary infection. It seems that at least in the antimicrobial or pre-antimicrobial era the fate of the individual child to develop meningitis or kidney tuberculosis was settled early. Also, those children, when followed up, developed what we call Simon foci. This is not only an academic question, ladies and gentlemen. We are spending a lot of time discussing preventive treatment of primary infection.

What I want to ask is this: What about preventive treatment in cases of obsolete or so-called obsolete hematogenous tuberculosis? What about preventive treatment of a patient who has calcified Simon foci? We know enough of the natural history of the disease to suspect that in many of those cases there are foci in the kidney or in the bones which might later explode; or a case of bone tuberculosis which for one reason or another did not get sufficient antimicrobial therapy.

I do feel that perhaps we should apply even more extensively than we do now, in our practical management of problems of childhood tuberculosis, our knowledge (which is already considerable) of the natural history of the disease.

DR. SCHAEFER: Dr. Cocchi wishes to say that he has treated his cases of bone tuberculosis by intra-articular injection of isoniazid and streptomycin, with very good results.

DR. MIDDLEBROOK: Does he not get good results without this?

DR. SCHAEFER: Inferior results.

DR. STRÖM: The question of the importance of trauma in the development of skeletal tuberculosis is very interesting. According to the literature, it is not quite solved.

We have tried in Stockholm to make a study of the importance of the trauma by using the method of isotopes, and in quite a large series of tuberculous animals, controls, and BCG-vaccinated animals we have performed trauma by breaking the leg of the animal under anesthesia and then giving it a bandage of plaster.

When in the X-ray picture it was noted that there was a callus, we injected labeled tubercle bacilli, labeled BCG, and examined the uptake in the different tissues and organs, and we found that in the site of the lesion in the animals which already had tuberculosis (it was inoculation tuberculosis) there was a significant increase of uptake of the labeled bacilli at the site of previous trauma. The difference was significant compared to the controls; but in the BCG-vaccinated animals there was no significant uptake or increased uptake of the radioactive bacilli or BCG as compared to the controls and as compared to the other parts of the skeleton.

Another difference was that in the traumatized tuberculous animals the development of callus was significantly less than in the controls.

I think this study, which is not yet published, could perhaps contribute to the question of the importance of trauma. We were not able to let the guinea pigs live as long as would be necessary to develop local tuberculosis; as a matter of fact, in none of the cases, even in the tuberculous cases, in which we found in the section manifestations of tuberculosis in other organs (mainly the spleen) did we find any development of tuberculosis at the site of the traumatic lesion.

DR. C. CHOREMIS (Athens, Greece): We think that a factor promoting the appearance of tuberculous ostitis, as it has been shown by laboratory data from our clinic, is the invasion of the bone marrow by the tubercle bacilli: thus, the frequently positive bone marrow cultures obtained in cases of the primary complex and tuberculous meningitis.

DR. RAFFEL: This perhaps turns a bit to the academic, but I have always been confused in the situation that Dr. Lack described, which has been well known for a long time.

We think of bacilli from possibly an earlier, now dormant, lesion, reaching, perhaps coincidentally, an area which has undergone trauma. In superficial terms, since trauma is obviously something detrimental to the body, we tend to think it is a perfectly logical sequence.

Actually, when you get down to the terminology employed in the last several years (and I stand to be corrected on this), it seems to me that those local conditions which exist in the traumatized area are among those that are felt to be more inhibitory rather than stimulatory for the growth of tubercle bacilli.

For example, the local blood supply might be interfered with to a certain extent, so that a relatively anaerobic condition might persist. Local acidity may be increased due to deterioration of normal metabolic processes. The low pH; the low oxygen levels; all those things supervene which a number of people now have shown to be really detrimental to the growth of the bacilli. Therefore, might we not just as reasonably expect the opposite effect.

DR. TERPLAN: Dr. Lack has touched upon a whole host of most interesting problems of internal dissemination and bone dissemination. I want to ask one question concerning something which impressed me in the material that we have examined during the last twenty-five years.

We have seen skeletal tuberculosis post mortem, usually in fairly close association with other manifestations of hematogenous tuberculosis in the face of a still-active lesion, a primary complex, or spreading primary complex in the lung. But one thing that impressed me was that there seemed to be a selective involvement of the vertebral bodies within the thoracic cage more frequently, let's say, than in bodies outside of the thoracic cage.

I wonder, inasmuch as Dr. Lack has such a tremendous experience, which I am sure no other pathologist in bone pathology has, whether he has seen anything similar. The only reason I ask the question is that we always try to prove or disprove whether there is some direct spread between lesions in the intrathoracic cage to the bones by the well-known and proved existing venous anastomoses, between, let's say, the venous system and the abdominal cavity and the venous system in the thoracic cavity and the epidural plexus.

DR. LACK: Fortunately from this point of view we have practically no information. We have only about two deaths a year, and so it is quite impossible for me to answer Dr. Terplan's question. I just haven't studied enough material.

## Session 4

Thursday Afternoon, November 10, 1955

ALBERT GUGGENHEIM, *Honorary Chairman*

LEE FORREST HILL, *Moderator*

# SOME OBSERVATIONS ON THE UTILITY OF SIMIAN PULMONARY TUBERCULOSIS IN DEFINING THE THERAPEUTIC POTENTIALITIES OF ISONIAZID

L. H. SCHMIDT[1]

### INTRODUCTION

In the past, systematic studies on the biology and chemotherapy of human tuberculous infections have been handicapped by the lack of suitable experimental counterparts, particularly for the various types of pulmonary disease. Recent studies have shown that there are striking similarities between both naturally acquired and experimentally induced pulmonary tuberculosis in the rhesus monkey, and the more fulminating forms of tuberculosis encountered in nonimmune and hypersusceptible human subjects. Collateral studies have shown that the induced simian infection can be manipulated systematically, in a manner which cannot be approached in the most extensive human studies, to give data which are important to a fuller understanding of both the biologic features of tuberculosis and the potentialities of new and old chemotherapeutic agents. Thus it would appear that the simian test object could be a useful addition to other experimental tools for tuberculosis research.

In the present report an attempt will be made to show how this simian test object has been utilized in defining the therapeutic and prophylactic potentialities of isoniazid. In achieving this objective, discussion will center largely on the first experiment on simian tuberculosis carried out in our Institute, a study designed to determine the capacity of isoniazid to control naturally acquired pulmonary tuberculosis in the rhesus monkey and to prevent dissemination of the disease from infected to noninfected nonimmune monkeys. Although more recent investigations on induced pulmonary infections have given more precise characterizations of certain of the therapeutic potentialities of isoniazid, none has dealt more effectively than this original study with both the therapeutic and prophylactic properties of this drug. The design of this therapeutic-prophylactic experiment is outlined in table 1.

The experimental material consisted of a group of 127 tuberculin-hypersensitive rhesus monkeys with roentgenographic and physical evidence of moderate to far advanced pulmonary disease, and a group of 51 tuberculin-negative apparently healthy animals. Similar numbers of each group of animals were divided among eighteen cages and the entire popu-

[1] The Christ Hospital Institute of Medical Research, and the College of Medicine, University of Cincinnati, Cincinnati, Ohio.

TABLE 1

OUTLINE OF STUDIES ON THERAPEUTIC AND PROPHYLACTIC ACTIVITIES OF ISONIAZID

Experimental Material:
- 127 tuberculin-hypersensitive monkeys with roentgenographic evidence of pulmonary tuberculosis
- 51 tuberculin-negative monkeys

Period of Isoniazid Administration:
- Similar numbers of infected and noninfected monkeys grouped together in each of 18 cages
- Isoniazid administration started April 28, 1952, terminated April 28, 1953:
  - 5 mg. per kg. from April 28, 1952 to July 7, 1952
  - 20 mg. per kg. from July 8, 1952 to April 28, 1953
- Measurements of tuberculin hypersensitivity and roentgenographic examinations at two-month intervals
- Sacrificing representative monkeys for bacteriologic and histopathologic studies on July 7, 1952, October 8, 1952 and April 28, 1953

Post-Treatment Observation Period:
- Separation of previously infected and noninfected monkeys into isolated cage groups
- Measurements of tuberculin hypersensitivity and roentgenographic examinations at two-month intervals
- Terminal bacteriologic and histopathologic studies
  - Sacrificing animals in either therapeutic or prophylactic groups at first roentgenographic evidence of new or reactivated pulmonary disease
  - Sacrificing all other animals between February 4, 1954, and November 19, 1954

lation was given isoniazid at a daily dosage of 5 mg. per kg. This amount, chosen on the basis of then available data on effective and tolerated doses, was fed in the diet: half at 7:30 a.m., half at 4:30 p.m. This regimen was initiated on April 28, 1952. Roentgenographic studies, carried out after two months of treatment, indicated extension of tuberculous lesions in about 20 per cent of the monkeys. This observation and the loss of 8 animals from overwhelming infections, together with supplemental information on the toxicity of isoniazid for the monkey, led on July 8, 1952, to an increase in the isoniazid intake to a daily dose of 20 mg. per kg., a level which was maintained for the remainder of the treatment period. Representative monkeys were sacrificed for purposes of histopathologic and bacteriologic study prior to this increase in dosage, and again after three months' exposure to isoniazid at the 20 mg. per kg. level. Roentgenographic studies and measurements of tuberculin hypersensitivity were made at two-month intervals.

Approximately one year after the start of isoniazid treatment, a third group of monkeys was sacrificed for bacteriologic and histopathologic purposes. Isoniazid was then withdrawn from the diet, and the infected or previously infected and noninfected monkeys were separated and caged in areas where the opportunities for cross infection between groups were minimal. Roentgenographic examinations and measurements of tuberculin hypersensitivity were repeated at two-month intervals. Animals were sacrificed whenever there was evidence of new or reactivated disease or at the end of the observation period, which fell between February 4 and November 19, 1954.

Before turning to the results of this study, attention should be drawn to the characteristics of untreated naturally acquired pulmonary tuberculosis in the rhesus monkey. Although previous experience in our Institute and elsewhere had indicated that the disease was highly fatal and readily disseminated, it seemed advisable to investigate these points under the conditions which prevailed in our

TABLE 2

OUTLINE OF STUDIES ON THE COURSE AND DISSEMINATION OF PULMONARY TUBERCULOSIS AMONG UNTREATED RHESUS MONKEYS

Experimental Material:
- 4 tuberculin-hypersensitive rhesus monkeys with roentgenographic evidence of pulmonary tuberculosis
- 39 tuberculin-negative monkeys

Observation Period:
- Similar numbers of noninfected monkeys placed in each of four cages with one infected monkey
- Measurements of tuberculin hypersensitivity and roentgenographic examinations at two-month intervals
- Sacrificing monkeys in terminal state for bacteriologic and histopathologic study

own colony at the time of the therapeutic-prophylactic study. To this end, the experiment outlined in table 2 was undertaken.

Four tuberculin hypersensitive monkeys with roentgenographic evidence of pulmonary disease were placed in four different cages among which 39 tuberculin-negative monkeys were distributed. Measurements of tuberculin hypersensitivity and roentgenographic studies were made at two-month intervals. When a terminal state was reached, the animals were usually sacrificed in the interest of obtaining the best material for bacteriologic and histopathologic studies.

* * *

*Nonimmune animals:* The data obtained from this investigation of the transmission of tuberculous infection from infected to nonimmune monkeys have been summarized in table 3. The summary shows that within twelve months of first exposure to infected monkeys, all 39 of the nonimmune animals had acquired tuberculin hypersensitivity. The survival times of 36 of the total group of 43 are summarized in table 4, which shows that approximately 75 per cent of the monkeys died within six months of the first objective sign of infection and that no animal survived twelve months. A significant fraction of the subjects, 20 per cent,

TABLE 3

TRANSMISSION OF TUBERCULOUS INFECTIONS FROM UNTREATED "CARRIERS" TO NONIMMUNE UNTREATED MONKEYS

| Date | Number of Monkeys with Tuberculin Hypersensitivity | |
|---|---|---|
| | At Start of Study | New Reactors |
| 4–28–52 | 4 | 0 |
| 7–24–52 | | 7 |
| 9–20–52 | | 5 |
| 11–15–52 | | 6 |
| 1–18–53 | | 4 |
| 3–25–53 | | 11 |
| 6– 2–53 | | 4 |
| 7–22–53 | | 2 |
| Total | | 39 |

TABLE 4

SURVIVAL TIMES OF MONKEYS WITH UNTREATED NATURALLY ACQUIRED INFECTIONS

| Survival After Development of Tuberculin Hypersensitivity | Survivors | |
|---|---|---|
| | Number | Per Cent |
| *months* | | |
| 0 | 36 | 100 |
| 2 | 28 | 78 |
| 4 | 16 | 44 |
| 6 | 10 | 28 |
| 8 | 5 | 14 |
| 10 | 3 | 8 |
| 12 | 0 | 0 |

succumbed within two months of the acquisition of hypersensitivity. The factor which determined survival appeared to be the development of demonstrable pulmonary lesions. The interval between acquisition of hypersensitivity and appearance of such lesions was extremely variable; yet, once pulmonary lesions were established, death invariably occurred within two to four months. An illustration of these events is given in Plates I and II, which record the roentgenographic findings in 2 monkeys which died three months after the first evidence of pulmonary disease, but three and eleven months, respectively, after the acquisition of hypersensitivity.

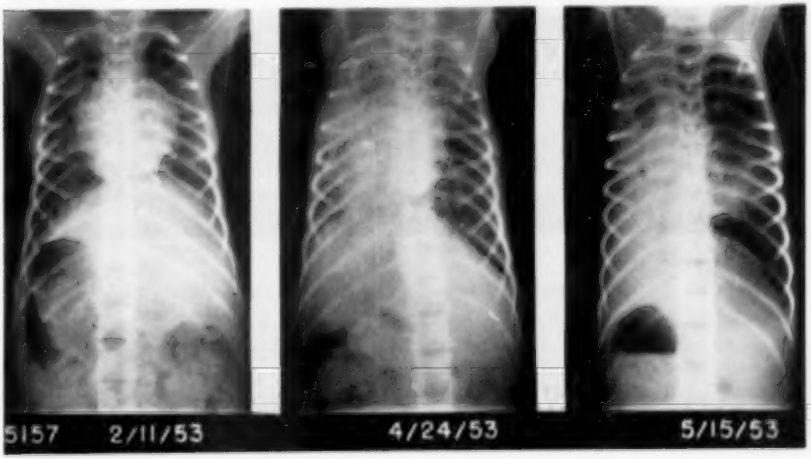


PLATE I

*Progression of Disease in Untreated Monkey 5157*

Tuberculin hypersensitivity first detected on February 11, 1953, at which time slight amount of infiltrate was noted in both right and left lung fields. Disease terminated fatally approximately three months later, on May 15, 1953.

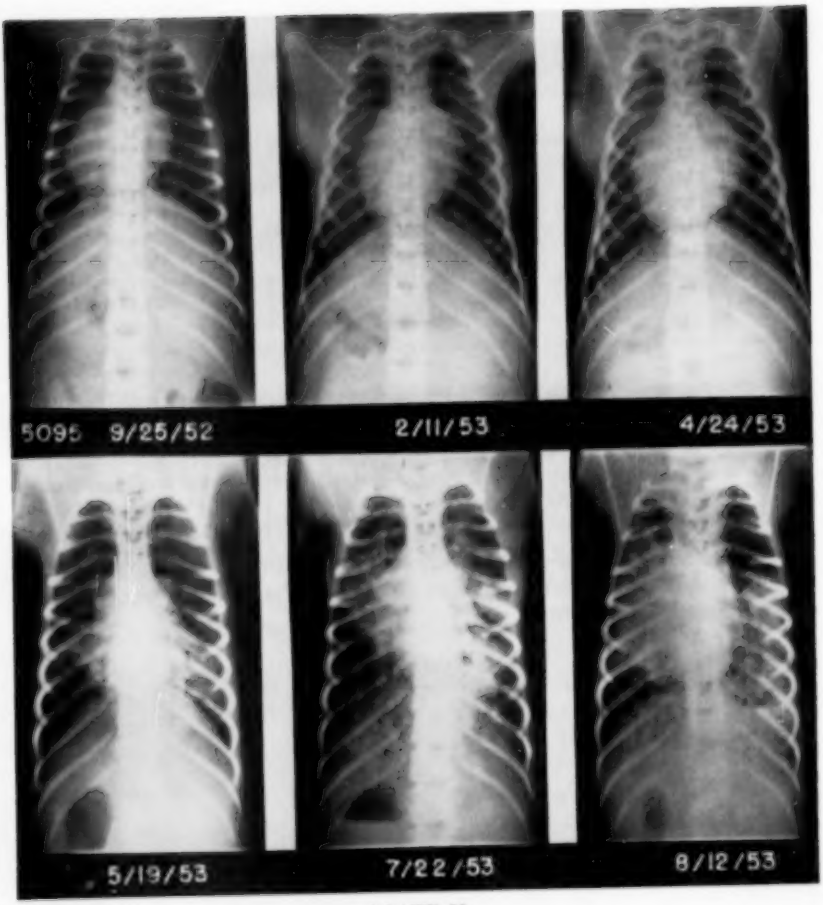


PLATE II

*Progression of Disease in Untreated Monkey 5095*

Tuberculin hypersensitivity first detected on September 25, 1952. Definite evidence of pulmonary infiltrate was not obtained until May 19, 1953. Death occurred approximately three months later, on August 12, 1953.

*Treated animals:* The sequence of events in the treated monkeys was strikingly different from that just described. As shown in column 3 of table 5, which summarizes the findings in treated animals with established infections, there were eight deaths from tuberculous infection during the first ten weeks of treatment in the interval when isoniazid was administered at a dosage of 5 mg. per kg. Only one additional death occurred during the remaining forty weeks of therapy with 20 mg. per kg. doses of isoniazid. Actually this fatality was the result of a non-

TABLE 5

THE THERAPEUTIC ACTIVITY OF ISONIAZID AGAINST ESTABLISHED PULMONARY TUBERCULOSIS IN THE RHESUS MONKEY

| Period* | Number of Monkeys Under Observation | Disposition of Group | | | |
|---|---|---|---|---|---|
| | | Number of Deaths from Tuberculosis | Number of Deaths from Other Causes† | Number Sacrificed | Number Remaining |
| 4-28-52 to 7-7-52 | 127 | 8 | 1 | 27 | 91 |
| 7-8-52 to 10-8-52 | 91 | 1+ | 1 | 18 | 71 |
| 10-9-52 to 4-28-53 | 71 | 0 | 2 | 16 | 53 |

* Isoniazid dosage: 5 mg. per kg. from April 28, 1952, to July 7, 1952; 20 mg. per kg. from July 8, 1952, to April 28, 1953.

† Result of intercurrent nontuberculous infection.

tuberculous complication associated with Pott's disease. It is clear, therefore, that, when administered at the 20 mg. per kg. dosage level, isoniazid provides a high order of protection against the usually fatal course of pulmonary tuberculosis in the monkey. As will be apparent from observations to be presented later, this broad protective action was associated with striking modifications of the roentgenographic, histopathologic, and bacteriologic pictures recorded in untreated tuberculous infections.

The sequence of events in the isoniazid-treated nonimmune monkeys exposed to infected treated monkeys was also strikingly different from that in untreated animals. As will be noted in the third column of table 6, no monkey which received isoniazid acquired tuberculin hypersensitivity during the treatment year, irrespective of the dosage regimen. Nor did any animal exhibit other evidence of tuberculous infection in this period. Since the acquisition of hypersensitivity by the monkey is a highly sensitive indicator of infection, it must be assumed either that infection had been prevented, or that, if it did occur, multiplication of tubercle bacilli was reduced to a level below that required to evoke and sustain a hypersensitivity reaction. In any event, it is clear that administration of isoniazid was highly effective in preventing the development of active infections in nonimmune monkeys exposed to highly infectious animals.

TABLE 6

THE PROPHYLACTIC ACTIVITY OF ISONIAZID

(Observations During the Exposure-Treatment Period, April 28, 1952, to April 28, 1953)

| Period | Number of Monkeys Under Observation | Disposition of Group | | | |
|---|---|---|---|---|---|
| | | Number of Tuberculin Reactors | Number of Deaths* | Number Sacrificed | Number Remaining |
| 4-28-52 to 7-7-52 | 51 | 0 | 3 | 14 | 34 |
| 7-8-52 to 10-8-52 | 34 | 0 | 1 | 3 | 30 |
| 10-9-52 to 4-28-53 | 30 | 0 | 1 | 2 | 27 |

* Result of intercurrent nontuberculous infection.

TABLE 7

FATE OF MONKEYS SUBSEQUENT TO TERMINATION OF ISONIAZID THERAPY OR PROPHYLAXIS

| At End of Treatment 4-28-53 | Number of Monkeys | | | | |
|---|---|---|---|---|---|
| | Sacrificed Prior to 2-4-54, Fulminating Disease | Sacrificed 2-4-54 to 2-15-54 | | Sacrificed 2-16-54 to 11-9-54 | |
| | | Active Tuberculosis | Negative | Active Tuberculosis | Negative |
| Therapeutic Group | | | | | |
| 53* | 3 | 1 | 40† | 1 | 6† |
| Prophylactic Group | | | | | |
| 27 | 6 | 7 | 9 | 0 | 5 |

* Two animals in this group currently alive, November 1, 1955.
† Either entirely negative or "inactive" residua.

*Post-treatment outcome in treated animals:* The post-treatment fate of monkeys in both the therapeutic and prophylactic series was a matter of special interest. In this area, follow-up observations were made on 53 previously infected monkeys and 27 noninfected exposed animals. As shown in table 7, these animals were followed for periods up to eighteen months after termination of treatment, the great majority for slightly more than nine months. In the therapeutic series, recrudescence of old infections was encountered in 5 monkeys, roughly ten per cent of the group. One animal, exhibiting slow spread of disease during the last three months of treatment, deteriorated rapidly after termination of therapy and was sacrificed in a terminal state two months later. The other 4 animals were sacrificed at the first indication of reactivation of disease, 2 after six months of observation, one after ten, and one after fifteen months. Forty-six other monkeys in the therapeutic series were sacrificed after observation periods of nine to eighteen months. Although in some cases scarring and other residua of tuberculous infection were found in the lungs and tracheobronchial lymph nodes, none of these animals presented gross evidence of active disease.

*Post-treatment outcome in animals given prophylactic isoniazid:* In the prophylactic series, the situation differed markedly. Thirteen of 27 monkeys, roughly 48 per cent, developed tuberculin hypersensitivity four to eight months after withdrawal of isoniazid. Within two months of this event these animals presented roentgenographic evidence of pulmonary disease and were found to have active tuberculosis when sacrificed. This high attack rate deserves comment, for it might be viewed as a consequence of cross infection and thus be unrelated to the stated objective of this study. This possibility does not appear likely in view of the simultaneous appearance of new infections in four isolated cages and the absence of such a phenomenon in our general monkey colony. It seems more likely that the disease which developed during the observation period had its origin in small numbers of tubercle bacilli which were present in the host at the termination of prophylaxis. Such organisms may have been acquired either early

or late in the prophylactic period. In the presence of isoniazid they were unable to multiply to the level required to provoke a hypersensitivity reaction; however, they were able to persist in the nonimmune host. Once administration of isoniazid was halted, they evoked a sequence of events similar to that which follows the induction of experimental pulmonary infections with small numbers of tubercle bacilli.

*Roentgenographic findings:* At this point it would seem of considerable interest to relate the terminal pathologic findings to the general therapeutic responses noted in the infected monkeys. Before proceeding with this item, however, a brief summary of the sequence of changes in pulmonary lesions observed in serial roentgenographic examinations is in order, for this sequence has to be understood in order to appreciate the histopathologic findings. As noted earlier in this discussion, administration of isoniazid at a dosage of 5 mg. per kg. for two months effected little change in the roentgenographic picture of some 60 per cent of the infected animals. About 20 per cent showed extension of the lung lesions; and a similar fraction, slight to marked clearing. The direction of these changes bore no relation to the amount of disease present at the beginning of therapy. It appeared from these findings that isoniazid was not able to effect rapid resolution of pulmonary infiltrate at a dosage of 5 mg. per kg., a conclusion which has been confirmed in subsequent experiments on induced pulmonary infections.

In contrast to the above observation, the roentgenographic findings after administration of 20 mg. per kg. doses of isoniazid for an additional three months were striking. At the end of this period dramatic clearing of the chest lesions occurred in every monkey, and there was a return to a normal picture in 52 of the 89 animals available for study. Cavitation was noted in 16 of the remaining 37 monkeys. For the most part these cavities were characterized by thin walls and absence of infiltrate in the surrounding tissue. Subsequent studies revealed a steady increase in the number of animals with roentgenographically normal pulmonary structures. At the termination of isoniazid treatment, cavitary lesions were noted in only 7 of 69 monkeys; marked residual scarring was seen in 6 other animals. One monkey, noted earlier in this report, exhibited a renewal of disease activity.

Examples of the sequence of roentgenographic changes observed in infected monkeys which exhibited typical responses to treatment with isoniazid are shown in Plates III and IV. The roentgenographic changes in the one treatment failure are shown in Plate V.

*Histopathologic findings:* Before turning to the histopathologic findings in the isoniazid-treated monkeys, a few comments on the pathology of untreated infections are in order. This untreated disease was characterized by a fulminating, progressing, primary type of tuberculosis. Large portions of the lung showed a caseous coagulation type of necrosis, the central zones of which were frequently undergoing softening. Cavitation within these caseous areas was common. These cavities were characterized by ragged linings within which acid-fast bacilli were numerous. In many instances, the caseous foci were surrounded by rims of granulomatous inflammation, irregular in outline and bordered with productive

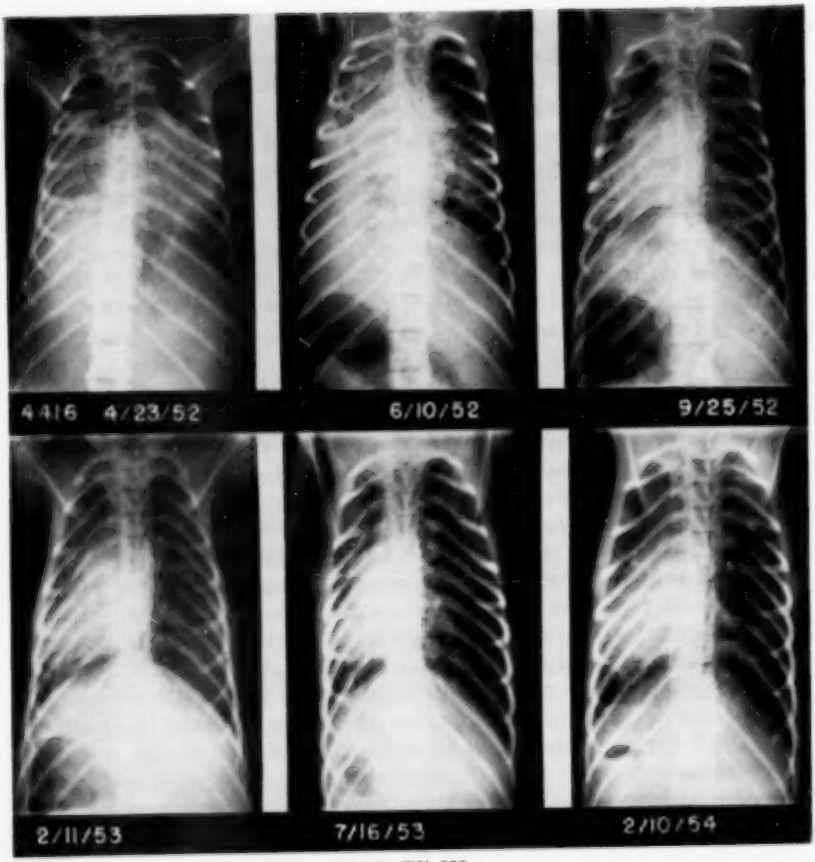


PLATE III

*Course of Disease in Treated Monkey 4416*

Status of disease prior to treatment with isoniazid (April 23, 1952), control of disease during treatment period (April 28, 1952, to April 28, 1953), and stabilization of control for ten months post treatment (to February 10, 1954). Isoniazid dosage: ○ 5 mg. per kg. daily, from April 28 to July 5, 1952; 20 mg. per kg. from July 5, 1952, to April 28, 1953. Note extension of disease with 5 mg. dosage.

tubercles. In other instances, the major caseous foci were surrounded by rapidly spreading, pneumonia-containing focal areas of caseation. In such cases, massive enlargement of the tracheobronchial lymph nodes with caseation was an almost universal finding. The gross lesions in a group of monkeys with untreated infections are shown in Plate VI.

The picture just described was in striking contrast to that encountered in the group of 16 animals sacrificed at the end of twelve months of treatment. All of

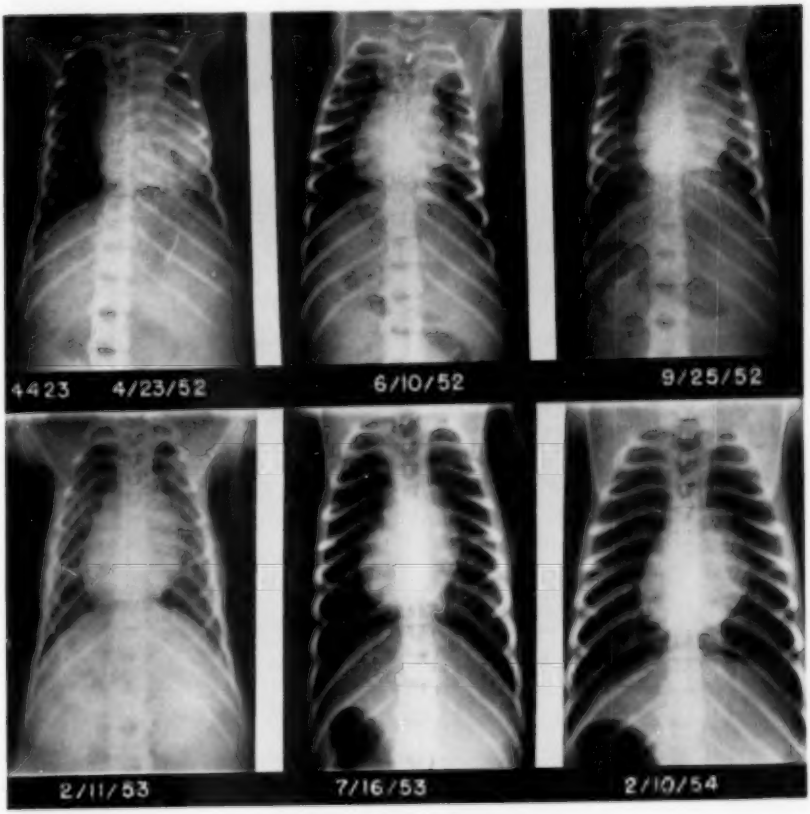


PLATE IV

*Course of Disease in Treated Monkey 4423*

Status of disease prior to treatment with isoniazid (April 23, 1952), control of disease during treatment period (April 28, 1952, to April 28, 1953), and stabilization of control for ten months post treatment (to February 10, 1954). Isoniazid dosage: ∞ 5 mg. per kg. daily from April 28 to July 5, 1952; 20 mg. per kg. from July 5, 1952, to April 28, 1953. Note regression of disease with 5 mg. dosage.

these animals had shown roentgenographic evidence of tuberculous infection prior to treatment; yet at necropsy 8 exhibited no evidence of intrathoracic disease. Histologically the only evidences of previous infection were interstitial fibrosis, chronic emphysema, and cystic bronchiectasis. Six other monkeys in this group exhibited residua of scarring at necropsy and presented histologic evidence of tuberculous infection which was thought to be stable. In 2 of these animals the lesions were limited to tracheobronchial lymph nodes which contained small caseocalcareous nodules completely surrounded by dense, collagenous connective

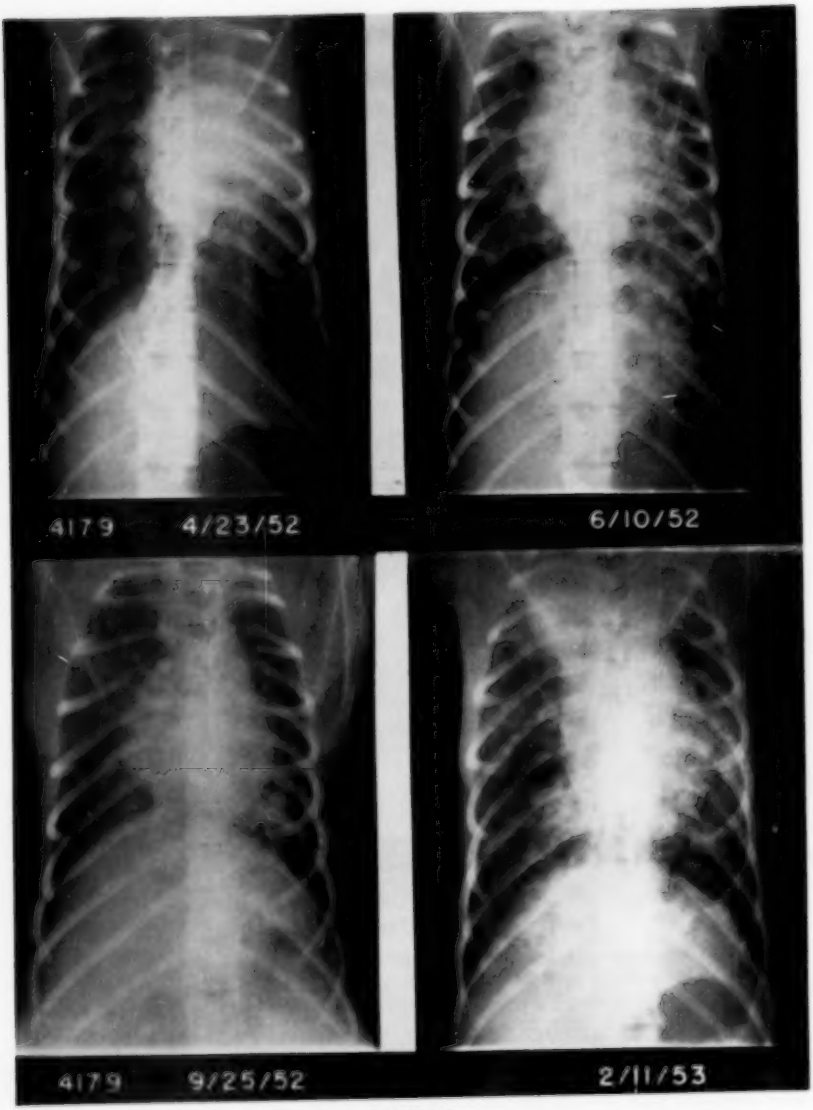


PLATE V

*Course of Disease in Treated Monkey 4179*

Status of disease prior to treatment with isoniazid (April 23, 1952), regression of disease during period of 5 mg. per kg. dosage (April 28 to July 5, 1952), continued regression during first segment of 20 mg. per kg. dosage period (September 25, 1952), and recrudescence of disease in latter phase of this period. Note massive nodal involvement in upper mediastinum (February 11, 1953). Death from overwhelming tuberculosis occurred approximately two months after the termination of treatment.

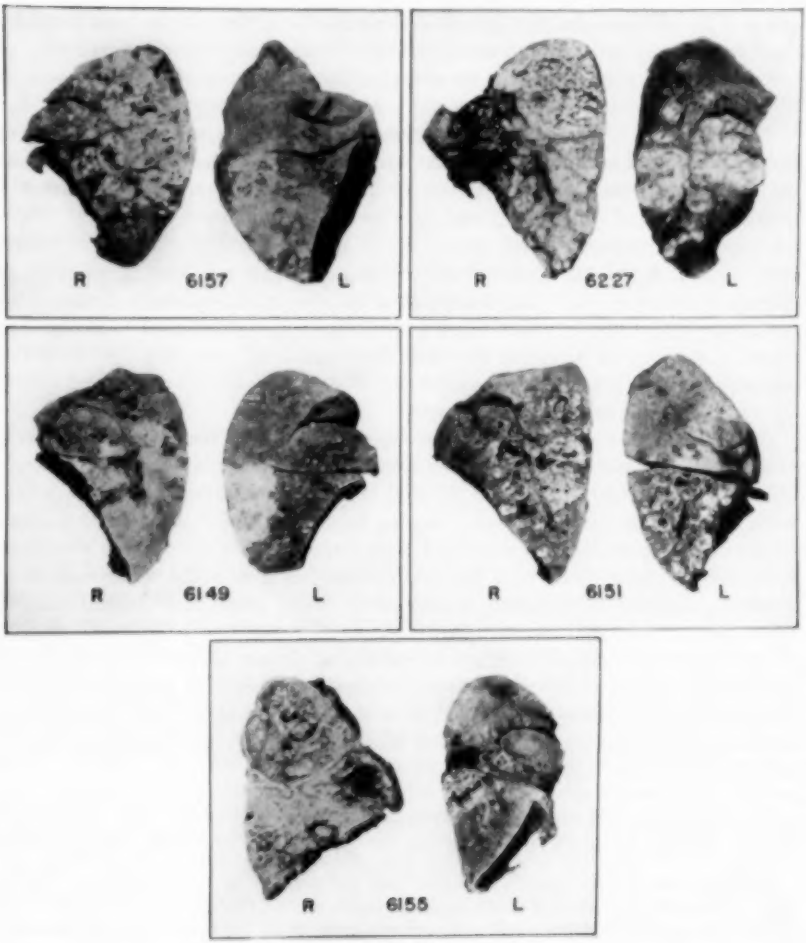


PLATE VI

*Lung Lesions in a Group of Monkeys with Untreated Infections*

Cross sections of right and left lungs of animals with fatal disease.

tissue. Two other animals among this group of 6 had fibrocaseocalcareous nodules in both lungs and mediastinal nodes, whereas the remaining 2 had lesions limited to the lungs. In one, the only evidence of previous infection was an occasional Langhans' giant cell associated with lymphocytic infiltration. In the other animal, several tubercles were found in zones of marked interstitial fibrosis and bronchiectasis. These were found in an area which on previous roentgenographic examinations had contained a 1.5 by 3.0 cm. cavity. The last 2 animals of the

group of 16 had open cavities. In one of these monkeys, the cavities were partially lined with metaplastic squamous epithelium; in the other, there was evidence of spread of the disease. With the exception of this latter monkey, enlargement of the tracheobronchial lymph nodes was conspicuously absent.

The above findings, the complete absence in many cases of any evidence of previous tuberculous infection, and the small inactive residua of infection in most other subjects, were also characteristic of the observations on the larger group of monkeys sacrificed nine to eighteen months after termination of therapy. These findings have been confirmed repeatedly in our Institute in subsequent experiments on induced pulmonary tuberculosis and have not been duplicated in infections treated with streptomycin or other antituberculous drugs. This histopathologic picture, which other workers have also associated with isoniazid therapy, appears to be one of the most distinguishing features of this drug and probably rests in large measure on the rapidity with which the compound arrests newly developing tuberculous processes.

*Bacteriologic findings:* Before terminating discussion of the therapeutic effects of isoniazid, it is appropriate to draw attention to the results of the bacteriologic phases of this study. It will be recalled that representative infected monkeys were sacrificed at various intervals during treatment and that all but 2 animals of the follow-up group were sacrificed after termination of treatment. Numerous specimens of lung tissue and tracheobronchial lymph nodes were excised at these necropsies, the number of specimens taken from each animal ranging from four to ten. The sites sampled included those in which there was obvious evidence of either active infection or residua of disease and, in some instances, apparently normal tissue at the locus of previous disease as determined roentgenographically. These specimens, as well as those taken from the infected untreated monkeys and those which had received isoniazid prophylactically, were treated in the following manner:

The samples were homogenized in approximately three volumes of sterile 0.85 per cent sodium chloride in a Ten Broeck grinder. Aliquots of the homogenate were inoculated directly into guinea pigs. Other aliquots were digested with alkali, sedimented by centrifugation, and the sediment was neutralized and planted on both American Trudeau Society (ATS) and Dubos Tween® albumin liquid media. Guinea pigs were tuberculin tested at four-week intervals and were sacrificed either at the appearance of disease or six months after inoculation. Splenic tissue taken aseptically from these pigs was homogenized, as described above, and cultured on the above media without digestion. All negative cultures were discarded after three months' incubation. Positive cultures were stored at 4° C. for later determinations of the susceptibility of the tubercle bacilli to isoniazid. These determinations have been carried out within the past six months, utilizing the 7H9 oleic-acid medium and the quadrant-plate technique developed by Middlebrook and associates.

At this time it is possible to deal with only a small fraction of the observations made during the course of these studies. In table 8 is summarized the recovery of tubercle bacilli from untreated monkeys and from those animals which received isonizaid prophylactically or therapeutically and were sacrificed at the end of treatment or later. This summary shows that tubercle bacilli were

TABLE 8

RECOVERY OF TUBERCLE BACILLI FROM UNTREATED AND TREATED MONKEYS

| Status of Donor Monkeys | Number of Monkeys | | Per Cent Positive for *M. Tuberculosis* |
|---|---|---|---|
| | Total Examined | Yielding Tubercle Bacilli* | |
| Untreated Controls | 34 | 34 | 100 |
| Therapeutic Series† | | | |
| Sacrificed 4–28–53 after 12 months of treatment with isoniazid | 18 | 5 | 28 |
| Sacrificed between 4–28–53 and 2–4–54 with active disease | 4 | 4 | 100 |
| Sacrificed between 2–4–54 and 11–19–54‡ | 47 | 10 | 21 |
| Prophylactic Series† | | | |
| Sacrificed prior to 2–19–54 with progressing disease | 13 | 13 | 100 |

* Either by direct culture or guinea pig inoculation.

† Monkeys in both therapeutic and prophylactic series received isoniazid from April 28, 1952, to April 28, 1953: 5 mg. per kg. for two months; 20 mg. per kg. for ten months.

‡ Only one monkey with active disease in this group.

isolated from all untreated monkeys and from all animals in either the prophylactic or therapeutic series which were sacrificed within ten months of termination of treatment because of development of active disease. On the other hand, bacilli were isolated from only 28 per cent of the animals in the therapeutic series which were sacrificed at the end of treatment, and from only 21 per cent of those sacrificed during the ten- to eighteen-month follow-up period; one of the latter animals had active disease. Striking as this comparison is, it does not give a wholly accurate picture of the bacteriologic situation in the diverse groups. It is somewhat more impressive to note that tubercle bacilli were isolated from more than 95 per cent of the specimens taken from the 51 monkeys with active disease, whereas fewer than 25 per cent of specimens positive for *M. tuberculosis* were derived from the 15 monkeys in the "terminal treatment" and "follow-up" groups. Despite this reduction in loci of viable tubercle bacilli, the fact remains that, in a comparatively incomplete survey of thoracic cavity structures, 9 of 46 monkeys harbored viable bacilli ten to eighteen months after apparent control of the disease. This finding, although in apparent accord with numerous observations on human pulmonary tuberculosis, is disquieting and "highlights" the problems of eradicative or truly curative therapy.

The data on the isoniazid susceptibilities of tubercle bacilli isolated from various groups of monkeys have been summarized in table 9. This summary shows that only 3 of the 26 monkeys in the untreated control group and only one of 11 in the prophylactic group yielded bacilli which could grow in media containing 0.1 $\gamma$ of isoniazid per ml. Quantitative assessments of the susceptibility of the culture population showed that less than 0.2 per cent of the organisms isolated in these 4 cases were able to grow in the presence of 0.1 $\gamma$ of isoniazid.

TABLE 9

ISONIAZID SUSCEPTIBILITY OF TUBERCLE BACILLI RECOVERED FROM UNTREATED MONKEYS AND THOSE RECEIVING PROPHYLACTIC OR THERAPEUTIC TREATMENT

| Highest Concentration of Isoniazid in Which Growth Occurred | Number of Monkeys Yielding Tubercle Bacilli | | |
|---|---|---|---|
| | Untreated Group | Treated Groups | |
| | | Prophylactic | Therapeutic |
| *γ per ml.* | | | |
| 0 | 26 | 11 | 8 |
| 0.1 | 3 | 1 | 5 |
| 1.0 | 0 | 0 | 5 |
| 10.0 | 0 | 0 | 3 |
| Total | 29 | 12 | 21 |

These findings point to two obvious conclusions: first, that the tubercle bacilli disseminated in our animal quarters at the time isoniazid treatment was initiated had a high order of susceptibility to this drug; and second, and more important, that this high order of susceptibility was not altered by the prophylactic use of the compound for a period of one year.

The data on the isoniazid susceptibilities of tubercle bacilli isolated from treated infected monkeys differed significantly from those just described. Thus, 13 of the 21 monkeys in the infected treated group yielded bacilli which grew in isoniazid concentrations of 0.1 γ per ml. or greater, 8 in concentrations of 1.0 γ or 10.0 γ per ml. Seven of the latter 8 "resistant" cultures were isolated from monkeys sacrificed at the end of twelve months' treatment or in the follow-up period. Only one animal sacrificed during treatment yielded resistant bacilli. The organism, a yellow bacillus, completely resistant to 10.0 γ of isoniazid per ml. of medium, was isolated from a caseous tracheobronchial lymph node of a monkey which had been sacrificed after ten weeks' treatment with 5 mg. per kg. doses of isoniazid. This animal had a solitary pulmonary lesion which roentgenographically had shown no response to treatment.

It is noteworthy that 2 of the 7 monkeys which yielded tubercle bacilli resistant to 1.0 γ of isoniazid were among the 5 which had reactivation of old lesions subsequent to termination of therapy. In both these cases, cultures isolated from multiple sites of pulmonary and nodal involvement exhibited similar responses to isoniazid; however, in all instances, only 10 per cent of the culture population was able to grow in 1.0 γ of isoniazid. It may be, therefore, that these so-called "resistant" bacilli did not play a major role in the reactivation of the disease.

None of the other 5 treated monkeys from whom isoniazid-"resistant" tubercle bacilli were isolated presented evidence of active infections. In 3 of these subjects, bacilli were isolated from well-circumscribed caseous foci in tracheobronchial lymph nodes; in the other 2 animals, the organisms were obtained from inspissated caseous material in small cavities. In each of these cases the culture population was completely resistant to 1.0 γ of isoniazid but only partially resistant to 10.0 γ of the drug.

* * *

Although time does not permit full discussion, it would be unfortunate to conclude this report without commenting on the striking changes in tuberculin hypersensitivity which occurred during isoniazid treatment of the infected monkeys. Throughout therapy there was a steady loss of tuberculin hypersensitivity until at the end of twelve months' treatment approximately 40 per cent of the animals were tuberculin negative. Reversions continued in the follow-up period until at the end of the study there were only 12 per cent positive reactors.

In summary, it is to be noted that an attempt has been made to show how naturally acquired or induced pulmonary tuberculosis in the rhesus monkey can be utilized for delineating the potentialities of new therapeutic agents. In doing this, attention has been focused on isoniazid and how it has been possible in a relatively brief period to define the broad therapeutic and prophylactic properties of the compound, its general dose-effect relationships, its rate of activity in terms of roentgenographic clearing of lung lesions, its discrete effects on healing processes, its ability to effect bacteriologic control, and finally its capacity to prevent relapse. Other studies which could not be reported here have dealt in a definitive manner with the activities of streptomycin, isoniazid, cycloserine, and combinations of these agents. It is believed that the information acquired in such studies is of a different order from that provided by other experimental infections and could be of tremendous assistance in defining both the attributes of new drugs and the mode of administration required to achieve most effective results. On the basis of this conviction, it is suggested that serious consideration be given to carrying out controlled simian studies regularly prior to the introduction of new agents for the treatment of human tuberculosis.

## GENERAL DISCUSSION

DR. HILL: Thank you very much, Dr. Schmidt. This paper is open for discussion. Who wants to start the ball rolling? I am sure there will be a great deal of discussion over this very significant and very important paper.

DR. MIDDLEBROOK: There is one point about this kind of work that I think should be emphasized. When Dr. Schmidt presented some of his data in Atlanta last winter, some individuals asked how much a monkey costs. Dr. Schmidt said thirty-five dollars. I think this question was asked in order to emphasize the possibility that you might, with a larger number of animals that are cheaper, be able to obtain information which would be perhaps a little bit more statistically secure.

But I would like to point out that actually in the running of any laboratory, or institute, only a small percentage of the total cost actually is determined by the cost of materials, animals or otherwise. It is labor that costs, and I would immediately like—before anyone asks the price of a monkey—I would immediately like to express my opinion that this is really a basically nonpertinent question so far as experimental investigations in animals are concerned.

DR. MCDERMOTT: I am always awed by Dr. Schmidt's reports because they are so lavish in so far as material for discussion, consideration, and reflection are concerned. I can only comment on a few things which impressed me about his work.

One is a broad generality, if you will. We talked a great deal yesterday on the subject of host resistance. We also have talked a great deal today on the point that antimicrobial drugs are not eradicative.

Here he has shown us a population of monkeys which are obviously highly susceptible, on the one hand, and which, on the other hand, receive a noneradicative drug therapy. When the drug treatment stopped, they still must have bacilli in them, or many of them have. Yet only 10 per cent relapse, which just sharpens up the subtleties of host resistance. Obviously, these monkeys do have a considerable degree of resistance which we cannot appreciate when we are seeing them overwhelmed by a first infection.

Now, of course, the most beautiful part of these studies, to my way of thinking, is the high percentage of monkeys which developed tuberculosis after a period of prophylaxis. That—it seems to me—is a very important point, which fits in well with everything we know about the problem of chemotherapy; namely, that there is something about an infection evolving to a certain point which makes it ever so much easier to control with the chemotherapeutic agents than when one gives the agent before the infection has evolved to that point. I think that this idea and these results of Dr. Schmidt's are rather in conflict with the results in the guinea pigs from Atlanta. I suspect the observations with monkeys are more comparable with the human situation.

This is not to say, however, that the Atlanta studies are not of the greatest importance and will not, in their subsequent development, lead to a great deal of clear-cut information. Finally, I would just like to talk about the question of the size of the dose of isoniazid.

Dr. Schmidt has been pushing for a high dose, and Dr. Middlebrook in the work here in Denver at the National Jewish Hospital, likewise, has been pushing for a larger dose of isoniazid.

I must say I am weakening. Any dose is a compromise, though, and it all depends upon where one wishes to use the drug. If one wishes to use the drug in carefully controlled hospital situations, then perhaps a large dose is justifiable.

Certainly, doses of 10 milligrams per kilogram or thereafter are toxic in humans, although only to a relatively few humans of any group. I have always believed that the great advantage of isoniazid was not the fact that it was another antituberculous drug, but an antituberculous drug that could be used on a largely unsupervised basis. From this viewpoint I would not like to see dosage increased so much. But this really becomes a question of ethics, indeed, as does the question of chemoprophylaxis itself. As for the true chemoprophylaxis of the Atlanta group and Dr. Schmidt's group—giving isoniazid to people who do not already have tubercle bacilli in their tissues—at the moment I feel there are very few situations in the world in which that would be justifiable. Certainly your results, Dr. Schmidt, would indicate that it is not too good an idea.

Dr. Canetti: I would just speak a little about the same problem, that of chemoprophylaxis, and the results which Dr. Schmidt has just now described.

I think we must be well aware that the species on which these experiments have been performed is quite different from what we have seen up to now, not only in man but even in experimental tuberculosis. This of course, alters entirely the conclusions we can draw from such experiments.

I will try to make myself better understood. Dr. Schmidt has told us that in the untreated, exposed control group, when he permitted exposure of the normal monkeys to tuberculous monkeys, if I understood well, after twelve months of contact, all the monkeys were dead. Isn't that so?

Dr. Schmidt: Not dead, but all infected. Some of them became tuberculin hypersensitive during the latter quarter of that twelve-month period, but tuberculin sensitivity was acquired by all of the exposed, nonimmune animals within a period of twelve months.

Dr. Canetti: Yes. May I make a comparison to what has been observed in another species of animal, the guinea pig?

Some years ago Dr. Max Lurie exposed guinea pigs to such a natural air-borne infection. We all know that guinea pigs are a very susceptible species, and we have assumed up to

now that no species is more susceptible than guinea pigs. When Dr. Lurie exposed his guinea pigs to natural air-borne infection from contact cases, from tuberculous animals in the same cages, after eighteen months he had only 36 per cent of his animals infected; that is, tuberculin positive.

Now, you see the difference between the two results. With one species, the monkey, you have 100 per cent infection during twelve months' exposure, and with the other species, guinea pigs, you have only 36 per cent infected during eighteen months.

This clearly points to an extraordinary susceptibility of the rhesus monkey to tuberculous infection. This, of course, has an important bearing on the problem of chemoprophylaxis.

The experiments of Dr. Schmidt show very beautifully that, while chemotherapy is being given to exposed animals, there is no tuberculin conversion—nothing happens. But, when the chemoprophylaxis is stopped, then in a certain number of cases—I don't remember how many—

DR. SCHMIDT: Fifty per cent.

DR. CANETTI: In 50 per cent of the animals, things go wrong: tuberculosis starts and many animals die.

Now this, of course, shows in a very definite manner that bacilli may have been inhaled by these animals, and chemoprophylaxis kept down their multiplication. As soon as prophylaxis is stopped, multiplication takes place. On the other hand, I think we should be aware that in human beings, if chemoprophylaxis were applied, there would be no reason whatsoever to believe that some inhaled bacilli during the period of chemoprophylaxis would begin to multiply as soon as chemoprophylaxis is stopped. It could perhaps happen in a few selected very susceptible persons, but not as it does in the monkey. So I believe that, while the experiments of Dr. Schmidt show very beautifully that bacilli are inhaled and are not killed during chemoprophylaxis, at least in this species, they do not permit us to draw conclusions as to what would happen in human beings. I would add, of course, in any case, that it would be important to confer on such animals a well-acquired immunity by BCG vaccination, as I have previously suggested.

DR. RAFFEL: I would like to comment on Dr. Canetti's comment.

I think the end result of what Dr. Canetti said, that implications or inferences cannot be drawn from one animal species and applied to another, is certainly well recognized, but I think this question of judging susceptibility of monkeys because of the high rate of conversion, 100 per cent conversion, on contact compared to 36 per cent in guinea pigs, is a rather simplified version of what constitutes susceptibility.

The guinea pig is a highly susceptible animal once the bacillus is in it, but is not as susceptible to infection as a human being. I think, perhaps, a very good analogy is given by the mouse.

The mouse, so far as I know, is completely insusceptible to the pneumococcus, if you leave the mouse in an environment where there are a lot of pneumococci. But put one or two bacilli into the peritoneal cavity of the animal and he is dead in thirty-six or forty-eight hours.

That portion of what you said needs some attenuation.

DR. CANETTI: I don't agree with Dr. Raffel. He said the guinea pig was not a susceptible animal with respect to getting infected. As soon as he has gotten infected, you said he is susceptible, but not for getting it. I must disagree. I think guinea pigs are extremely susceptible to tuberculous infection. But I think if you put, for instance, human beings—that is what interests us—in as infective an environment as you could imagine for eighteen months, you would never get 36 per cent of tuberculin conversions.

I think that this 36 per cent of tuberculin conversions observed by Dr. Lurie by exposing animals simply to natural air-borne infections is a tremendously high rate, and it shows very well that the guinea pig is a susceptible animal, not only once it has acquired infection, but to infection itself. The point I wanted to make is that Dr. Schmidt's monkeys are even more susceptible.

DR. SCHMIDT: As a pharmacologist, I have always worried about numbers, and I think both of us are talking about essentially uncontrolled experiments.

We do not know to how many tubercle bacilli our monkeys were exposed, and it is also true that in most of these other circumstances, when one puts guinea pigs or rabbits in areas where there is general dissemination of organisms, the inoculum is an essentially unknown thing.

I think that until we know the inoculum and until we know the susceptibility of the species via the same route to the same inoculum, we ought not to argue too much about differences in susceptibility.

DR. CANETTI: I think Dr. Schmidt is perfectly right, speaking about inoculum, but we shouldn't forget in air-borne infection, in natural infection, the differences of inoculum size are not as great as they may be in experimental injections by other routes.

There may be a difference, perhaps, from two- to tenfold but they do not vary as in injections by other routes; so that, while I very well realize that conditions of exposure certainly play a role, I don't think in such experiments, in which you have animals in cages, that there could be very big differences in exposure from one experiment to another.

DR. MIDDLEBROOK: I think there is one very great difference, Dr. Canetti. You know that monkeys cough and guinea pigs do not. The chances of a droplet nucleus of proper size being formed and being inhaled would seem to me to be much greater in the monkey-to-monkey transfer than in the guinea-pig-to-guinea-pig or rabbit-to-rabbit transfer. In the first situation, you have droplet nuclei formed from coughing. In the other case, you have infection from inhalation of particulate material stirred up in the cage. The droplet nucleus or dust particle has to be small enough (less than 5 microns in diameter) to be inhaled and to initiate an infection. It seems much more likely to me that a droplet nucleus would be of proper size than a dust particle.

This is one aspect of the problem with which we should be concerned, as well as with the possibilities of different degrees of susceptibility to infection.

DR. DAVIS: I would like to ask Dr. Schmidt a question. Were the monkeys separated from the infected monkeys and was isoniazid continued for any period after the monkeys were separated? That would have a bearing on the outcome of the experiments, it seems to me.

Did you stop the isoniazid and separate the monkeys at the same time or continue the isoniazid two or four weeks afterwards?

DR. SCHMIDT: That is one of the weak points of the experiment. If you read the fine print, I said the infection might have been acquired either late or early during the course of the exposure period. Treatment was terminated at the same time the animals were separated.

DR. HILL: While you are on your feet, I am sure some of our clinicians are immediately trying to transfer your experiments over to the human being. Your dosage of 5 mg. per kg. did not eliminate tuberculosis from the animals but 20 mg. did. Now, in human beings as I recall, we have been using only 5 or 10 mg. of isoniazid per kg. in tuberculous meningitis. One wonders whether we now ought to increase our dosage in man. It will be understood that way if published in newspapers anywhere. What can we think or do or decide about animal experimentation of this sort in immediately transferring it over to the human being?

DR. SCHMIDT: I wonder if I could address myself to that question in a form which was not planned, but which I have yearned to do ever since I saw the program. There has been nothing in the program on the metabolic disposition of isoniazid in different subjects, and yet, I think it is one of the most important aspects of chemotherapy. It is actually just about as important as the *in vitro* susceptibility of the organisms in the infected individual.

Isoniazid is extensively degraded in man and extensively degraded in the monkey—more extensively in the monkey than in *most* human beings. The antimicrobially active moiety is *free* isoniazid. It then becomes important to understand what concentration of free isoniazid is attained in animals on diverse doses.

In the monkey a dose of 5 mg. per kg. gives a trough level of free isoniazid in the blood

between 0.7 and 1.2 mg. per ml. A dose of 20 mg. per kg. of isoniazid gives a concentration of the order of 2.5 to 3.5 in the trough when the total dose is divided into two equal doses per day. Eighty milligrams per kilogram dosage, which is actually the dose we found gave maximum therapeutic response, will give a minimum concentration of free isoniazid of the order of 6 to 9 $\gamma$ per ml. of serum during the interim between doses.

Now, it takes a much smaller dose of this drug to achieve these same therapeutic levels in man. On the other hand, we have reason to believe that some men are going to behave like monkeys. Others will almost behave like dogs, and a dog and a monkey represent the two extremes in so far as the degradation of this drug is concerned. Therefore, I think that it is important to determine whether those individuals who are not getting a good therapeutic response have adequate concentrations of free isoniazid in their blood. It may be that the poor therapeutic response in some patients is due to very low blood levels of microbiologically active isoniazid.

DR. MIDDLEBROOK: May I ask you if you don't think it would be important to know *before* the patient doesn't do well what kind of blood levels are being achieved?

DR. SCHMIDT: I think you are right, Dr. Middlebrook.

DR. IRVINE: May I ask two straight questions on a subject which isn't a specialty of mine. I was called in by the London Zoo to observe tuberculosis amongst their monkeys and they got hopelessly lost in my advice. The thing that I should rather like to know is what tuberculin test Dr. Schmidt uses, because I injected astronomical doses into the eyelid and got no results at all, and had to fall back on the BCG test, which, incidentally, worked very nicely. Another question: Is Dr. Schmidt interested in calcification in monkeys' lungs by X-ray?

DR. SCHMIDT: The answer to the first question is that we use tuberculin (OT) in dilutions varying from 1:10 to 1:10,000. We do not use the palpebral fold test for determining tuberculin hypersensitivity. We draw a large cross on the abdomen and inject four different concentrations, 0.05 ml. of each of these four concentrations into the abdomen. In that way we obtain an indication of the peak allergic state which is reached—which permits a quantitative assessment of changes which occur during the course of therapy.

We have seen calcifications many times in treated monkeys, but have never seen them in the untreated because this is too fulminating a disease to allow calcification.

DR. MCDERMOTT: I would like to ask a question, and also issue a soft reproach.

The question is, have you ever seen roentgenographic relapse during therapy with isoniazid? The soft reproach has to do with dosage. In that, I think—well, I can speak only for myself—I think it is dangerous to say that some maximal therapeutic response is provided by such and such dosage when the only indices are comparative roentgenographic measurements. Speed of roentgenographic clearing, on the one hand, and cultures taken relatively late in the course of the disease, on the other, lead to a somewhat cloudy situation. But my basic question is about relapse under treatment.

DR. SCHMIDT: We have seen evidences of relapse under treatment in only one animal in this particular series. We have done several times as many experiments with induced infections as we demonstrated here today—apparent remission, and then relapse under treatment, is a fairly regular occurrence with 5 mg. per kg. doses.

DR. MCDERMOTT: The relapse disappears?

DR. SCHMIDT: Oh, no.

DR. MCDERMOTT: Despite change in therapy, it goes right on.

DR. SCHMITT: The relapse occurs during therapy. Now, your reproach is, I think, well justified, but perhaps it can be tempered by the fact that we observe a rather regular time sequence of changes in the roentgenographic picture under the impact of different doses of isoniazid. We also observe terminal bacteriologic pictures which vary with different doses of isoniazid. And, third, we have a histopathologic picture which suggests whether or not in any animal the disease continued for a considerable period of time before there was a significant arrest of the infectious process. On high doses of isoniazid there was a uniformly rapid arrest of the process, without exceptions.

Dr. McDermott: I do accept the difference that you have shown between the results on 20 mg. and 5 mg. per kg. dosages. I simply wanted to make the point—I don't think there is much hope that we would do better in humans than we can now do at 10, if we could give 50 mg. per kg. per day.

Dr. Middlebrook: Well, Walsh, I don't know whether I have told you about our case which best illustrates this problem in the human subject. I will use initials. MH was treated with isoniazid at 16 mg. per kg. per day and with streptomycin, 1 gm. a day, for two months before we noted there was nothing significant happening to the bacterial population in her sputum. She was still excreting only isoniazid-susceptible bacilli. It is true enough there was a decrease in amount of sputum. I won't mention that aspect of the problem, but this was the only patient out of 20 followed in similar fashion who failed either to convert to negative within two months or to get some isoniazid mutants appearing. For this reason we did blood levels of isoniazid on this patient MH. According to previous studies we found the six-hour level, after the standard dose of 4 mg. per kg., in patients on a total of 8 mg. per kg. per day to be rarely less than 0.4 $\gamma$ of isoniazid per ml. of serum by microbiologic assay. At six hours, by microbiologic assay, there was less than 0.2 $\gamma$ of isoniazid per ml. in this patient's serum after an 8 mg. per kg. dose! By the usual Kelly and Poet method there were 6 mg. of isoniazid per ml. of the same serum, at least a thirty-fold difference there. And Major Morse was kind enough to do a chemical assay by Cuthbertson's picryl chloride method and he found there was less than —?

Major Warren Morse (Denver, Colorado): One-tenth of a microgram per ml.

Dr. Middlebrook: Less than one tenth of free isoniazid. And yet, this patient among at least 40 is unique. She gets good blood levels at two hours—one to two hours after the standard dose. But, immediately thereafter it disappears and it isn't just being excreted rapidly because there were the isoniazid derivatives in the serum as determined by the Kelly and Poet method.

I think Dr. Schmidt and I are talking about the marked individual differences from patient to patient.

There may be two approaches. One can give large doses to everybody; but, as you pointed out, Dr. McDermott, on the scale in which you have long envisaged the great possible usefulness of isoniazid for tuberculosis to be so striking, so remarkable, your hopes may be defeated by this pharmacologic phenomenon in individual cases. If one has them in the hospital, one might use a standard large dose, but I am not sure that even the 16 mg. per kg. will always be adequate. It wasn't adequate for patient MH.

But, at least, one could say either use a high dose on all patients; or you might say, let's do microbiologic assays on each and every patient.

I must say we haven't done enough microbiologic assays, in general, to predict precisely what will result statistically. I can only say that, of 20 patients who developed resistant mutants which were all catalase negative, 18 of them had higher than the average blood levels among a large series of unselected cases. On the other hand, we have encountered 3 patients who were on 8 mg. per kg. per day from the beginning of the treatment—and one, at least, on 16 mg. per kg., patient MH—who had very, very low serum levels compared with the average of the distribution curve. This is as much as we can say at the present time and I think really what one should do is study serum levels in hundreds of cases and do careful, pharmacologic documentation in order to determine just what the statistical significance of the whole phenomenon is. Maybe you don't mind "losing" one patient out of 20. Dr. Dressler says he minds very much losing one patient out of 20. That might be the result of a peculiar attitude at this hospital. Maybe from the public health standpoint, a person would say one is justified in applying standard lower dosage if one can take care of 95 per cent of cases; one can forget about the other 5 per cent.

Dr. McDermott: Mr. Chairman, may I simply say I quite agree with Dr. Middlebrook and Dr. Schmidt. Indeed, I used to hit Middlebrook over the head with Schmidt, but now they seem to be ganging up on me!

Dr. Mitchison: May I ask Dr. Schmidt one question, whether his evidence provides

something on the question of infectivity of the isoniazid-resistant strains? Has he got any idea of what the bacterial populations were in terms of the proportions of susceptible and resistant organisms during the presumably most highly infectious period, the first five or six weeks of treatment of the already diseased animals?

DR. SCHMIDT: The answer to that is simply that we do not have the information, that the bacteriologic work was done at a time when the most we could do was to obtain the organisms either from infected guinea pigs or from direct culture of the monkey tissues, and then go ahead and determine the susceptibility later. Obviously, that practice provided no opportunities for determining what proportions of the organisms are susceptible or resistant during the early treatment period.

DR. MITCHELL: Has there not been a series of cases, Dr. Schmidt, studied for degradation of isoniazid? Is it not more frequent than one in 20 rapidly metabolizing isoniazid?

DR. SCHMIDT: Dr. Biehl, who is sitting here in the audience, is actually implementing this study, which at the present time involves almost 100 patients. Among these, one finds a whole range of rates of metabolic inactivation of isoniazid. To the present time, most of our data have been acquired on urinary excretion and one finds some individuals at the bottom of this scale who degrade isoniazid the most—who excrete only 1.5 per cent of the total dose of drug in the free form. On the other hand, the excretion of this moiety will run up to 40 per cent of free isoniazid in those who metabolize the drug to the least degree.

DR. STEAD: I heard you say, Dr. Schmidt, there was some correlation between toxicity and the amount of free isoniazid in the blood. This you mentioned in commenting on dogs having such a great amount of free isoniazid in their blood. It harked back to the fact we were all quite cautious about isoniazid when it first came out because of its great toxicity in dogs, and subsequently found out that in other animals this didn't turn out to be true. Is there a correlation here?

DR. SCHMIDT: There are data already published on the point you have raised, Dr. Stead. Admittedly, they are limited, but work which was done by Dr. Biehl and one of my colleagues at the Institute, Dr. Hughes, has shown a direct correlation between the occurrence of peripheral neuritis and the amount of free isoniazid excreted in the urine.

There is no question that the dog is a poor destroyer of isoniazid. Just as the dog has a very limited ability to acetylate the sulfonamides, so he has essentially no ability, at least, to excrete isoniazid as the acetyl derivative. Free isoniazid is more toxic than acetyl isoniazid by a factor of 16. It is more toxic than isonicotinic acid by a factor of 50. Isonicotinic acid is one of the other major excretory products.

# TUBERCULOUS CUTANEOUS COMPLEXES IN CHILDREN

R. V. PLATOU[1] AND R. H. LENNOX[1]

## INTRODUCTION

Few clinical areas are more beclouded with confusing concepts and cumbersome classifications than is that of tuberculosis involving the skin. Although variations in the actual presenting manifestations are certainly wide, reading and experience convince us that almost all cases can be conveniently fitted into one of four meaningful categories. It is our purpose here to justify this attempt at simplification by means of a brief review and description of several pertinent examples.

While several stages of any one type or combinations of more than one type of cutaneous tuberculosis might occur in the same patient simultaneously or at different times, the following four patterns seem reasonably discrete: (*1*) direct inoculation, (*2*) hematogenous seeding, (*3*) direct extension from an adjacent lesion, and, finally, (*4*) a rather heterogeneous group considered particularly to reflect hypersensitivity.

Despite the fact that most forms of cutaneous tuberculosis present highly characteristic—often pathognomonic—features, writings on the subject have been relatively sparse in this country, and in most of our clinics at the present time tuberculosis of the skin is considered to be distinctly rare (1). Certainly, adequate information has not yet been recorded to combine with our experiences for expressing a meaningful opinion concerning the efficacy of modern therapeutic agents for these forms of the disease per se.

## TYPES OF DISEASE

### *Direct Inoculation*

Primary infections generally present rather uniform characteristics, almost regardless of site of inoculation. The major variations are due perhaps to coincident or complicating local and systemic conditioning factors, such as infection with other organisms. The portal of entry through the skin or mucous membrane appears typically as an indolent small papule or granuloma, often crusted or ulcerated, which causes remarkably little symptomatology or concern except that it persists for a much longer period than one would expect following ordinary abrasions, scratches, or puncture wounds (2). Within a month or so, there is conspicuous regional lymphadenopathy progressing to caseation, the size of the latter usually bringing the patient to medical attention.

At about this same time, tuberculin hypersensitivity develops, so that, with knowledge of such conversion in this sequence, the diagnosis can usually be made with high assurance that histologic and cultural confirmation will follow (3).

These changes, so easily seen on the body surface, undoubtedly mirror the

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much more common pulmonary pattern following first inhalation of tubercle bacilli. The changes thus always impress the student with the relatively insignificant local lesion which is to be contrasted with the huge regional reaction in the lymph nodes.

Typical cases of primary skin tuberculosis have been described which follow a wide and interesting variety of causes of disruption of the various body surfaces. In all of these cases, the disruption of the skin is presumably accompanied or soon followed by invasion of tubercle bacilli from contaminated dust, droplet, instrument, or weapon. Apparently, any or all types of minor or major wounds have been the basis for clinical notes! For obvious reasons, particular interest has been attached to those following circumcisions (4), piercing of earlobes (5), swimming-pool abrasions (6, 7), vaccinations or injections (8–10), various irritative eruptions (11), bites (12), and so on, through a seemingly endless variety of circumstances; all of these cases have the common features of some break in the skin, together with direct or indirect contact with tubercle bacilli.

While it is possible that tubercle bacilli may invade directly through intact conjunctival or mucosal surfaces (13), they rarely ever gain access through the normal skin under ordinary conditions (14). On moist surfaces, such as the lips, genitalia, or perianal areas, local lesions may develop a striking superficial resemblance to those of syphilis, so that it is not surprising to find many descriptions of "tuberculous chancres" in older writings (15).

Although much has been written and said about the typical "apple jelly" appearance of local nodules, these are not particularly useful in distinguishing between primary inoculation and spread of tuberculosis from endogenous sources. When tuberculous lesions in the skin are compressed, as with a glass slide, tubercules then show through the epidermis or overlying inflammatory reaction as rather discrete and sharply circumscribed brownish or reddish-yellow masses, 1 to 2 mm. in diameter (16).

Constellations of these may be found to make up varying patterns within almost any type of lesion. When they occur in crops and clusters, they are considered particularly typical of lupus vulgaris, an entity which is nearly always secondary, occurring in previously sensitized persons, who have manifest tuberculosis elsewhere than in the skin.

In certain circumstances there may be a violent local hypersensitive reaction at the site of reinoculation with tubercle bacilli, Koch's phenomenon. But it seems adequately clear that, after many or most naturally acquired cutaneous reinfections in children, lesions are much more indolent and regional lymphadenopathy is much less conspicuous than with first infections (3).

It hardly seems necessary to recall that the type or intensity of reaction at the site of reinfection or elsewhere will vary with the number of organisms, their virulence, and also the allergic state of the host. However, there seems to be general agreement with our experience that reinoculation in sensitized persons results most frequently in tuberculous warts: "tuberculosis verrucosa cutis," "verruca necrogenica," "butcher's wart," "pathologist's wart." These are

usually sharply localized, chronic, and rarely accompanied by much significant regional lymphadenopathy or systemic effects.

Even these warts are unusual, and it is our belief that many or most reinfections produce little more than the reaction expected with ordinary positive routine diagnostic tuberculin tests. We could find only one case we considered an exception to the more usual benign course following reinoculation into a sensitized host. The following brief protocols illustrate, first, the classical sequence expected with primary inoculation of the skin; and, second, an example of a severe reaction following reinoculation of a sensitized child.

Sam R., a Negro boy of ten years, was admitted to the hospital in 1951 for study of a large slightly tender mass in the left groin which had gradually increased in size over a period of about three weeks. He appeared otherwise well and, because a surgeon thought that the mass was of inflammatory nature and fluctuant, it was incised; however, this yielded nothing. Then a more careful examination disclosed a scabby dry plantar sore, adjacent to the ball of the left foot, and a number of small yellowish nodules were seen when this was compressed with a glass slide.

The boy didn't remember how he got this wound, and had complained to no one about any pain or particular discomfort in his foot. A Mantoux (OT, 0.1 mg.) was positive, although this reaction had been negative elsewhere six months earlier.

Roentgenograms of the chest were clear, but tubercle bacilli were soon seen and cultured from discharge which appeared in the wound after a few days. Therapy with streptomycin-PAS was then initiated, and the plantar lesion was excised. Sections of the latter revealed typical tubercles with abundant acid-fast organisms.

The wound in the foot healed uneventfully per primum, and the huge femoral, inguinal, and iliac adenitis resolved completely within five months. Seen recently, almost five years later, he appeared entirely well, with no deformity and barely discernible scars at the sites of previous incisions.

Although excision of the plantar lesion might not have been necessary to secure an equally good therapeutic result, we still believe that it is important to excise the primary focus or "feeder-lesion" whenever this procedure is practical and involves no great additional risk to the patient. The patient's course, which might have been the same, although more protracted, without streptomycin-PAS therapy illustrates the usual benign pattern following primary inoculation into the skin.

Joe R., an eleven-year-old Negro boy, was admitted to the hospital in June, 1955, because he had had large nodes in the neck for three years, together with a very sore right eye and an increase in neck swelling for about three weeks since his eye had been scratched with a stick while playing in his yard.

Six years earlier, in 1949, this child had been treated elsewhere for interstitial keratitis, presumably syphilitic in origin. At that time, a routine tuberculin test was strongly positive, and calcified hilar nodes were seen in roentgenograms of the chest. Treated with penicillin systematically, together with local compresses and atropine on the eye, the keratitis had resolved slowly over a period of about six weeks, and the patient did not return to the other hospital for follow-up care.

Among 14 members of this rural household were 10 persons known to have tuberculosis and 4 others who had refused skin tests or roentgenograms.

Examination disclosed an acutely ill boy with a violent keratoconjunctivitis, cloudiness of the anterior chamber, and severe photophobia prohibiting adequate examination of

the uveal tract or retina. There was a shallow ulcer over a cluster of tubercles just medial to the lacrimal gland in the right tarsal conjunctiva. Conspicuous preauricular swelling was noted, and massive matted and fluctuant cervical lymphadenopathy, particularly on the right side. Rupture of one mass of nodes seemed imminent, and aspirated material yielded morphologically typical tubercle bacilli on direct smear; cultures were confirmatory. Roentgenograms showed calcifications in enlarged hilar, peritracheal, and cervical lymph nodes. A routine skin test (first-strength PPD), inadvertently given by the intern, resulted in a central slough, with satellite lesions about this reaction which were considered morphologically typical of *lichen scrofulosorum*.

Within forty-eight hours after starting therapy with isoniazid (8 mg. per kg. daily in divided doses) and PAS (0.2 gm. per kg. daily), the irregular hectic fever subsided and remarkable subjective improvement occurred. Although a mild exacerbation of phlyctenular conjunctivitis occurred four weeks after therapy was started, the conjunctival ulceration and the cluster of tubercles continued to resolve and had disappeared by six weeks. General improvement continued, with satisfactory weight gain and increase in hemoglobin and gradual clearing of all evidences of systemic infection. While ophthalmologic examination at present is not entirely satisfactory, due to scars and opacities from two or more severe bouts of ophthalmia, progress is considered satisfactory. Cervical lymphadenopathy is still present, however, but it is relatively inconspicuous, with no discharge or skin defect.

The details of this child's illness six years earlier are not entirely clear, but it is certain that at that time he had a positive Mantoux test and calcified hilar nodes. When we saw him at the time of his present illness, he was acutely ill with a violent inflammatory reaction in the right eye, due probably to invasion of tubercle bacilli following a superficial injury to the tarsal conjunctiva.

Although a routine diagnostic tuberculin test was certainly unwise, this was done hurriedly before any information had been secured concerning his earlier hospitalization and diagnosis of tuberculosis, and it fortunately was followed by no recognizable worsening of symptoms or signs in the eye or elsewhere.

The cluster of satellite lesions in the skin adjacent to the superficial slough following this test probably represented sterile inflammatory perifocal lesions produced by diffusion of tuberculoprotein from the necrotic lesion into the immediate surrounding area.

Because this was the first time we had seen such satellite lesions accompanying even the most violent local reactions to tuberculin, these were biopsied and showed epithelioid reactions. Tubercle bacilli could not be recovered, even though therapy with isoniazid-PAS had been initiated about three weeks before these specimens were removed.

While information is wanting in the way of earlier records, we believe this was the best clinical example we have encountered of Koch's phenomenon, a severe focal reaction following reinoculation of tubercle bacilli into a sensitized person.

### *Hematogenous Seeding*

Although rare, in this country at least, the most familiar forms to illustrate this manner of spread are probably disseminated lupus vulgaris and discrete tiny tubercles in the skin accompanying other clinical evidences of miliary spread. Disseminated lupus is most apt to occur after a variable but long period

following infection and during the course of common exanthematous diseases of childhood, such as measles or chickenpox.

Ustvedt and Ostensen (17), in a sample of 167 cases, tabulated the intervals from assumed primary infection to the appearance of tuberculous lesions in the skin, kidneys, and adrenals. While their figures indicated that such intervals may be considerably shorter among persons who have their first tuberculous disease during or after twenty years of age, these differences were probably not significant. They concluded that "skin tuberculosis in the majority of cases is diagnosed considerably later after primary infection than pulmonary tuberculosis, and seems to be about in the same position with regard to the time interval as renal tuberculosis, whereas tuberculosis of the suprarenal glands mostly appears still later."

In contrast to reports from Norway, where lupus vulgaris appears to be a fairly common cutaneous form of tuberculosis, this lesion has been remarkably rare in our experience. In the best clinical illustration we have encountered, we could not establish a tuberculous etiology:

H. J., a three-year-old Negro boy, was admitted to the hospital in 1947 with classical clinical features of chronic basilar meningitis, comatose and with severe opisthotonos. He had been treated in another hospital over a period of five weeks for meningitis of unknown cause. The pattern in the cerebrospinal fluid was entirely compatible with a tuberculous etiology, but we could not isolate organisms, and tuberculin tests were repeatedly negative. As many other causes of chronic meningitis as we could think of were excluded.

Overshadowed by the severe symptoms and signs of apparently terminal meningoencephalitis, with a moderate degree of communicating hydrocephalus, this child had a number of raised lesions of varying sizes and in various stages of necrosis, but all were considered morphologically typical of lupus vulgaris. Biopsy of one of these lesions soon after it appeared showed only "acute and chronic dermatitis, with endothelial proliferation and new blood vessel formation."

Following a very hectic course of therapy which we would nowadays consider grossly inadequate, this child eventually made a remarkable recovery after a period of approximately four months, during which he was entirely comatose. Now, eight years later, he appears physically and neurologically intact. While we could not prove a tuberculous etiology for his disease, we could certainly not disprove it. His remarkable recovery from a long-continued, truly moribund state confirms our belief that there are yet no completely adequate criteria by which a specific diagnosis or accurate prognosis can be rendered in all cases of tuberculosis. The skin lesions gradually resolved over a two- to three-month period, and at present there are no discernible residua other than a small scar at the site of biopsy.

Like most other evidences of miliary tuberculosis, cutaneous manifestations are apt to appear relatively soon after the primary infection. These, in our experience, are both rare and inconspicuous. We have recognized typical extensive tubercles in only one young child among nearly a hundred with miliary and meningeal tuberculosis whom we have treated during the past twelve years.

I. K., an eighteen-month-old Negro girl, presented with an acute febrile illness. The history at admission indicated that she had been intimately exposed to open tuberculosis. Classical, clinical, and roentgenographic features of miliary disease were promptly revealed. Scattered widely over the body, but particularly numerous and clustered over the posterior aspects of the legs, were numerous tiny lesions which might easily be mistaken for low-grade folliculitis or "goose-pimples."

These were very firm, and on close inspection and under pressure consisted of tiny yellowish nodules, each less than a millimeter in diameter, with no apparent inflammatory reaction.

Although biopsies were not made, the involution of these lesions paralleled that of the typical miliary lesions in the lungs under therapy with streptomycin-PAS. This child, too, was treated before isoniazid was available, and we have seen no other example of apparent miliary tuberculosis affecting the skin either before or since.

Suffice it to say that, in our experience, cutaneous evidence of hematogenous spread of tuberculosis is rare and, when it occurs, it is overshadowed in importance by accompanying visceral lesions.

Whether appearing as lupus vulgaris or as scattered miliary tubercles, the prognosis and course appear to be essentially those of the more important visceral manifestation. Furthermore, it is often difficult or impractical to establish conclusively the tuberculous etiology of many such lesions which appear clinically to be a part of, or due to, hematogenous or lymphatic spread. Certainly one could imagine that there might be an exceptional patient in whom an exact etiologic diagnosis would depend heavily or exclusively on precise study and elucidation of the cause for its cutaneous manifestations. Such a tuberculous child we have not yet ourselves encountered.

### *Direct Extension*

Scrofula (scrofuloderma, scrofulous diathesis) is still, in our experience, a fairly common cutaneous manifestation of tuberculosis in children. The mechanism by which it develops is apparently by direct extension from an underlying or adjacent tuberculous focus, usually involving lymph nodes or bone.

Most commonly, the scrofulous diathesis is part and parcel of the clinical picture of progressive primary tuberculosis; these patients are usually obviously sick, presenting with poor nutrition, anemia, and reasonably typical signs of tuberculous disease.

With effective means now available for treatment—and perhaps even prevention—of these progressive primary infections, it is now relatively unusual to witness the chronic course, with long-continued discharge from one or several sinus tracts and the aftermath of disfiguring scars which formerly were so commonly encountered.

Even in neglected cases, when extensive necrotizing changes in the skin have occurred, with development of actively discharging sinus tracts, prompt response to therapy has occurred in those patients whom we have treated since the advent of streptomycin and isoniazid.

However, despite relatively early and prompt closure of sinus tracts and gratifying resolution of underlying lymphadenopathy, these scrofulous lesions have appeared to be very prone to late recurrences. Soon after the advent of streptomycin, we began to see pleasing results of various combinations of medical and surgical therapy, but we soon found that practically all of these children had local recurrences within a period of three years.

It is our practice now to treat these patients for at least three months after maximal resolution of all evidence of tuberculous disease has occurred, and then

continue PAS in conventional dosage for another full year. If tonsillectomy is indicated, this is deferred until maximal resolution has occurred, while continuing isoniazid-PAS. Certainly, if regional nodes are to be excised, we prefer that this operation be preceded by tonsillectomy also (18). So far, under this plan, results have been uniformly gratifying, but sufficient time has not elapsed for evaluation of enough cases to impress us that it is the "best" plan. Certainly with this manifestation, as with all others of active tuberculosis in children, we have learned with experience that therapy for each patient must be highly individualized. Following is a very brief description to illustrate some of the problems, variations, and gratifying early results of medical therapy directed at scrofula:

I. P., an eighteen-month-old Negro girl, on admission to the hospital presented classical evidences of progressive primary tuberculosis with miliary and meningeal involvement. Other conspicuous evidences were in the tonsils, huge caseating cervical nodes with bluish necrotic changes in the overlying skin, and an area of osteomyelitis at the tip of the mandible.

The Mantoux reaction was positive and gastric washings were positive for *M. tuberculosis*. Roentgenograms showed several large areas of consolidation, increased bronchovascular markings, miliary lesions, and huge hilar, peritracheal, and peribronchial nodes.

With supportive measures, isoniazid (8 mg. per kg. per day) and PAS (0.2 gm. per kg. per day), practically complete resolution of all manifestations except the mandibular osteomyelitis has occurred within a period of eight months. Excision of the mandibular lesion now appears to be indicated, and we plan to accomplish this some time in the next few months.

This sequence contrasts pleasantly with the far more protracted and unfavorable course expected prior to the advent of streptomycin and isoniazid therapy; selection of the proper time for excision of a favorably situated but unfortunate bone lesion remains our major problem. The relatively slow and poor response of osseous lesions influences us strongly now toward favoring a surgical attack. Certainly, this story is typical of the scrofulous diathesis as a part of progressive primary infection.

### *"Toxic" Reactions*

Most confusing and intriguing of the cutaneous manifestations of tuberculosis are those in which it is difficult or impossible to recover tubercle bacilli, leading to the inference that they represent a response of hypersensitivity.

Most of these, described under a seemingly endless list of eponyms and morphologic features, are characteristically indolent, appearing in crops in or about the follicles or nodes of intact skin, and are not accompanied by any conspicuous regional lymphadenopathy or constitutional reaction.

They tend generally to follow the pattern of visceral manifestations in the individual affected, and are apt to resolve spontaneously or recur quite unpredictably.

Apparently, some of the classical and morphologically distinctive lesions, such as papulo-necrotic tuberculids or very minute papules of lichen scrofulosorum, may also result from hematogenous spread, as is suggested by the occa-

sional discovery of organisms in histologic sections. Most of these, however, probably reflect a peculiar type of hypersensitivity to organisms situated elsewhere in the body. Minor variations are described as "folliclis," "acne scrofulosorum," "toxituberculides," "acnitis," "disseminated follicular lupus," and so forth. Of course, even when tubercle bacilli are not recovered from lesions of this type, it is possible that they had been present earlier, only to be destroyed in the skin either before the lesions were manifest or the biopsy was made.

Perhaps the most intriguing "ids" we have encountered were those which occurred about the site of a reaction to PPD in the child described earlier as an example of a violent focal inflammatory reaction following reinoculation (*Patient Joe R.*).

A large variety of patterns has been described under the heading of papulonecrotic tuberculids. Some time ago we had a unique opportunity to observe extensive lesions of this type in a child who presented no other clinical evidences of serious underlying tuberculosis.

M. W., a four-year-old Negro boy, was seen in the clinic for enuresis of apparently functional type. During the initial examination it was noted that there were a number of superficial irregular dry flat plaques up to 3 cm. in diameter scattered over his trunk and arms, which could best be described as resembling old necrotic reactions to multiple tuberculin tests.

These had first been noted by the mother about a month earlier and had apparently caused no symptoms or concern. There was no known contact with tuberculosis, and no source of infection was subsequently discovered. The child appeared entirely well. A routine hemogram, erythrocyte sedimentation rate, and several urinalyses yielded no abnormal findings.

A tuberculin test (OT, 0.1 mg.) produced a reaction in appearance practically identical with the lesions described earlier. There was no significant lymphadenopathy, and roentgenograms of the lungs showed nothing abnormal. Although permission for biopsies could not be secured, we believed that these large flat necrotic plaques constituted a variant from more typical papulo-necrotic tuberculids, occurring as manifestations of hypersensitivity in an otherwise "silent" primary infection with symptomatic measures. Nothing was done for treatment of the skin lesions, which disappeared gradually over a period of seven weeks.

While it is generally appreciated that erythema nodosum, in this country as contrasted with northern European areas, is a very rare cutaneous manifestation of tuberculosis, it too may occasionally occur as a manifestation of severe hypersensitivity. Among 5 children with typical erythema nodosum whom we have seen in recent years, 4 had conspicuous evidences of streptococcal disease and rheumatic fever, while the fifth one may have been the single example of erythema nodosum related to tuberculin hypersensitivity that we have encountered.

J. B., a white girl of eleven years, developed a number of painful, tender, red areas over the anterior aspects of both legs, six days before admission to the hospital. At first, the parents thought these were probably mosquito bites. They gradually grew larger, very tender, and the child was noted to be feverish.

The parents and four siblings were apparently in good health, and subsequent examinations of them disclosed no evidences of tuberculosis.

Examinations revealed only typical painful and tender large erythematous swellings

over the shins and knees. A moderate leukocytosis was observed and an elevated erythrocyte sedimentation rate (60 mm. per hour, Wintrobe); urinalyses showed nothing of note, and there were no direct clinical or bacteriologic evidences of streptococcal disease.

A tuberculin test (OT, 0.1 mg.) was strongly positive, roentgenograms showed nothing abnormal, and over a period of two weeks, with limited activity and occasional doses of salicylate, the lesions had largely resolved, while fever and other evidences of infection subsided.

During the next six years there have been no further evidences of active tuberculosis or rheumatic fever.

## DISCUSSION

Obviously, one would expect the incidence of the cutaneous manifestations of tuberculosis to be low wherever the incidence of the disease itself is low. Because the time which elapses from primary infection to the appearance of most cutaneous forms of tuberculosis is reasonably long, this low incidence would be particularly conspicuous in children. We were gratified just last month to find that in a sample of a thousand children under seven years, selected from the areas of our city known to have the highest rates for tuberculosis in adults, investigators from the Tuberculosis Division of the U.S. Public Health Service could find less than 1.5 per cent who reacted positively to patch tests and intracutaneous PPD in the first strength (19). In a recent survey of approximately 36,000 children of school age in 28 parishes of Louisiana, only 6.6 per cent reacted to tuberculin patch tests (20).

Because we see a large number of children at the large Charity Hospital, we see a wide variety and a considerable number of most diseases. From our experience, then, it was not surprising, despite the low incidence of tuberculosis, that we could find examples of most cutaneous manifestations on which to base our present attempt at simplification of a confused terminology. Perhaps the single example of note that we have not encountered is erythema induratum (Bazin). We have inquired of many colleagues and have found no one else in our area among pediatricians or dermatologists who has had a recent case to illustrate this manifestation.

Aside from those manifestations of tuberculosis resulting from direct inoculation and the forms (whether primary or reinoculation forms), which are usually the most typical and easily recognized lesions, tuberculosis affecting the skin is quite invariably and literally secondary. While the dermatologic patterns are almost innumerable and often most intriguing, our major concern as clinicians should be and usually is directed toward a search for the far more important underlying lesions. When these are found and properly attacked, experience teaches that most cutaneous manifestations will recede in a satisfactory fashion.

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## GENERAL DISCUSSION

Dr. Marianna Gardner (Denver, Colorado): I would like to ask Dr. Platou if these individuals with cutaneous tuberculous lesions have positive tuberculin skin tests in a high percentage of cases.

Dr. Joseph A. Johnston (Detroit, Michigan): I thought the incidence in skin tuberculosis ran parallel with the incidence of bovine tuberculosis. Certainly in Scotland, where bovine tuberculosis is high, it would run higher than in the United States.

My own impressions on this problem came from living in Boston (which was notorious at that time for never having done anything about bovine tuberculosis) and then moving out to a forward-looking area like Detroit, where milk had been accredited for twenty-five years.

Doug Chadwick happened to move at the same time as I, and he remarked to me, after about five years, on the almost complete absence of skin tuberculosis in the Middle West. With the exception of the cervical nodes, we could have given you a dozen examples of all of these other things in Boston, but we could not do it in Detroit.

Dr. Martin Fitzpatrick (Kansas City, Kansas): I would like to ask, in these patients with cervical adenitis, if he could determine the portal of entry, and in how many of them was the tonsil involved.

Dr. Platou showed us one case of a child having a skin inoculation from a family with a very heavy exposure. I wonder, in the other children with primary inoculation, whether they came from homes where there was heavy exposure or whether possibly they could have gotten the infection in school or from some other source.

Dr. Lincoln: With regard to Dr. Johnston's comment, I would like to point out that in New York we have had pasteurization for a great number of years, and we see all the varieties and a few more than Dr. Platou has described. We think it is more the portal of entry that determines scrofula than it is the type of organism.

I would also like to comment that we happen to have seen, a great many years ago, exactly the type of tuberculin reaction following the tuberculin test that Dr. Platou had, with a corona of tubercles around it. We were curious enough to do a biopsy and found exactly the same histologic reaction as he did.

Dr. Sifontes: I would like to agree with what Dr. Lincoln said. We don't have bovine tuberculosis in Puerto Rico, and we have the same experience as that of Dr. Platou.

I would like also to ask Dr. Platou about the pulmonary lesions in these cases that he has shown.

Dr. Kendig: I talked to Dr. Platou in the hall before this session started, and I told him we didn't see very much cutaneous tuberculosis in Richmond, but I do think we see a good bit of cervical node involvement.

We have just completed a study of twenty-five cases, and the question has come up as to exactly where the tuberculosis has come from. Of the eleven tonsils that were sectioned, only in two of them were we able histologically to find tubercle bacilli. The question comes up whether or not it is actually an extension from the tonsils or whether it is part of a hematogenous dissemination.

Dr. Eugene McEnery (Chicago, Illinois): Concerning erythema nodosum, Dr. Platou mentioned that in the Scandinavian countries it was usually followed by some form of pulmonary tuberculosis. I remember some time ago that Dr. Wallgren wrote an article about erythema nodosum associated with pleural effusion. He was so positive about it that he felt that, about three months after erythema nodosum occurred, pleurisy with effusion would result.

I wonder if some of our guests from the Scandinavian countries still feel that way about it. I know in our district we see quite a few cases of pleurisy with effusion, but we were never able to demonstrate any cases of erythema nodosum.

Working the other way, as Dr. Platou did, we have had numerous cases of erythema nodosum, but we never observed the child to develop subsequent pulmonary tuberculosis; it was usually associated with some rheumatic condition.

Dr. John Lichty (Denver, Colorado): We had a specific case of tuberculous involvement of lymph nodes in which we could find nothing significant in the throat. The child had had an ear pierced for an earring and there was a little chronic inflammation at the site of the piercing, even though it had been done several months earlier.

I would like to ask Dr. Platou whether he has seen any cases where it might have been trauma like that which would be the portal of entry.

Dr. Ström: As Dr. Platou mentioned, we see a certain percentage of erythema nodosum in our primary tuberculosis cases, in about 20 to 25 per cent of them, occurring regularly within the first three weeks.

I don't know if I misunderstood, but we don't see pleural effusion regularly in those cases in which we have observed erythema nodosum. There is no correlation between the two. However, if there is a pleural effusion, it occurs between three and six months after the primary tuberculosis; that is to say, when the children are getting tuberculin positive and have manifestations of a primary complex in the lungs.

Why we see this high percentage of erythema nodosum in the Scandinavian countries, we don't really know. There was an investigation a few years ago which showed that in

those cases there was a high titer of streptolysin in the blood serum: there seemed to be a certain correlation between a high sensitivity of the skin and a high streptolysin titer.

Dr. Grzybowski: On this question of erythema nodosum and pleural effusion, we were very interested in this problem because we were under the impression that pleural effusion might be more common in cases of primary tuberculosis with erythema nodosum than in cases of primary tuberculosis without erythema nodosum. We studied very carefully an admittedly small group of cases of erythema nodosum, and we found that the incidence of pleural effusion was equal in both groups.

Dr. Terplan: I think Dr. Platou, as far as I know, has the largest number of primary tuberculosis cases that I have ever heard of. In the older literature about the various portals of entry of primary tuberculosis, such findings were listed as exceptions. I remember one in the skin, in the conjunctiva, and then, of course, from very massive environments in the nose or occasionally, rarely, in the tonsil.

I think the most interesting picture he showed was the proof of a true reinfection complex in the presence of still persisting allergy or sensitivity to tuberculin.

Dr. Kassowitz: I would like to add one more source of primary skin infection which belongs to past history—injection of whole blood into the thigh, followed within two, three, or four weeks by a caseous lymphadenitis in the groin, verified as a transmission by way of whole blood injection.

Dr. Platou: I think the question concerning positive skin tests requires reference back to this matter of chronology and sequence of the development of the primary inoculation. Of course, in such cases, for the first three weeks after implantation, you watch for conversion to a positive skin tuberculin test.

The other type of tuberculosis in the skin, in which you are apt to have confusion with relation to skin tests, is the papular necrotic group. This is not a unique experience with us but, in talking with others and in reading, this is a group that defies the immunologist. The local lesion may have a perfectly typical structure, even with recovery of organisms, and so on, but with negative skin tests. These people with chronic papular necrotic tuberculosis are a rare breed.

In all of the other types you would expect (except in the small moribund group) to have positive skin tests.

I can't answer Dr. Johnston's question about bovine tuberculosis. I am sure it has been very important in the past, but in our cases we have made no effort to distinguish types of bacilli. There was only one out of the group in which circumstances suggested that the child probably had tuberculosis of bovine origin.

I don't know any way to prove it, but I think it is pretty well accepted that the common portals of entry for cervical lymphadenitis are two: lymphatic spread from the parenchyma of the lung, parahilar, paratracheal and on up, particularly with involvement of the central submental nodes; or directly from tonsillar infection. I think we have seen plenty of both of them.

As we are in a state of confusion regarding the treatment of caseating nodes of the neck in children—and I was told this morning that this is going to be an item for discussion on the agenda Saturday morning—perhaps it is just as well to skip that for the present.

I suppose the site of infection must be as variable as the number of square millimeters on the body surface; but the common sites were illustrated.

Concerning the question of associated pulmonary lesions, I don't have figures, and they would be meaningless on such a small series (I think it is about 50-50 with skin tuberculosis). About half the time you see obvious evidences of tuberculosis elsewhere, certainly with the severe cases giving evidence of hematogenous spread. This attests the point we made before in summary—that the important thing about skin tuberculosis, with the two exceptions cited, is to find the important lesion and treat that, and the skin disease will clear without much trouble.

In regard to Dr. Kendig's question, I think discussion of cervical lymph nodes had perhaps best be postponed for Saturday, when it is tentatively scheduled.

I am glad to hear about the current status of erythema nodosum in Sweden. As Dr.

Lichty's question intimates, piercing the earlobes for earrings is a common cause of inoculation tuberculosis. We went through the literature to find all other exotic mechanisms by which primary inoculation can be achieved, and the list is limitless. There are penicillin injections, injections of BCG (swimming pool abrasions of course are well known), and so on.

In answer to Dr. Terplan's remarks, I would like to reassure him that the cases shown today, while most of them come from our experience at Charity Hospital, don't all come from there. I had to scout around to find some of these illustrations. Dr. Miller furnished me with one, and Dr. Mickelson furnished me with others. I don't know of anyone who has a complete collection of all forms of cutaneous tuberculosis in his clinic. We missed erythema induratum; we couldn't find it. Dr. Mickelson sent me one, but I didn't show it because he said it was the best one he had and he didn't think it was good enough. It is a rare lesion.

Someone told me today that he now has a patient under treatment for erythema induratum, but we haven't seen one.

DR. TERPLAN: Except for some of the historically well-known observations on inguinal lymph nodes, up until 1930 we had only one single inoculation of primary tuberculosis in the skin. That was published because it was so rare in our clinic.

# NUTRITION AND TUBERCULOSIS IN THE ADOLESCENT

JOSEPH A. JOHNSTON[1]

In a study originally planned to gain information on the subject of endogenous versus exogenous reinfection, 932 tuberculin reactors, removed from contact and placed in foster homes of the Society of St. Vincent de Paul, were followed at six-month intervals for various periods up to twenty years. Thirty-nine reinfections were detected. Ten of these could not be considered to have developed endogenously, since the first films obtained showed evidence of an adult type of lesion. Twenty-one of the other 29 cases occurred in females and the lesions in these 12 developed within the span embraced by the period two years preceding and three years following the menarche.

For this reason, metabolic studies were planned on this age group with the following objectives: (*1*) to obtain information from nitrogen and calcium balance studies which would permit the inference that a depleted state had preceded the development of adult-type tuberculosis; (2) to determine the correlation, if any, of the course of the pathologic process with the nutritional state as reflected in the balance data.

As a background for these studies, data were accumulated on the metabolism of the normal adolescent. Included in the observations were the food requirements of adolescents and a variety of factors influencing food utilization, such as the effects of nontuberculous infection, hormones, physical activity, and rest.

Rich has summarized the status of the relationship of tuberculosis to adolescence as follows:

> The sharp rise in mortality that follows the childhood period of relative safety is associated with the following circumstances: (*1*) A progressive increase in the incidence of infection; (*2*) apparently a specific adverse effect of the state of pubescence on resistance; and (*3*) the beginning of exposure to the stresses and strains associated with a struggle for existence and with childbearing. That other less evident factors may be operative cannot be denied.

That the nutritional state of the adolescent girl in particular may play a role in reinfection at this age is stressed in the following quotation from the report of the League of Nations, although supporting data are lacking:

> . . . This is the age when the most serious cases of pulmonary tuberculosis occur. Every tuberculosis specialist is convinced that the appearance of tuberculosis before the twentieth year is due to two main causes, overwork and malnutrition.
>
> Although the general incidence of tuberculosis has been decreasing in many countries gradually during the past 100 years, and especially during the past thirty years, it is a distressing fact that this rate of reduction does not occur to the same extent in the case of adolescent and young adult people. Indeed, in young women there has been an increase in tuberculosis during recent years in some countries. This state of affairs has been ascribed by some to the modern habit of "slimming," by others to the greater expenditure dictated by modern habits of life on clothes, which leaves too little money

[1] Henry Ford Hospital, Detroit, Michigan.

for the purchase of proper food. Whatever may be the real explanation, it is undoubted that many adolescents are being improperly nourished at the present time and are suffering in consequence.

We have reviewed elsewhere the literature (1) on the studies attempting to relate nutrition and tuberculosis. A brief review will be presented here of a series of studies on the normal adolescent, followed by examples of the patterns of retention of nitrogen and calcium in patients with lesions showing healing without specific treatment other than rest and diet, patients with unstable or "spreading" disease, and patients treated with streptomycin. Some general remarks will be made on these studies at this point and the individual case studies will be presented at the end of the paper.

Adolescence was shown to be characterized by two types of metabolic processes: in the pre-pubertal period with an accelerated rate of growth, a relative increase in the basal metabolic rate, and an enhanced capacity to retain nitrogen and calcium; in the post-menarcheal period, a marked deceleration in the rate of growth and a diminished ability to retain nitrogen and calcium. On the average, the menarche occurs when the decelerative process has been operative for about a year. The fall in nitrogen and calcium retention in the post-menarcheal period may be interpreted merely as a function of the diminishing rate of growth; but its abrupt nature, followed in some instances by a temporary recovery (plus the added demonstration of the depressed retention induced by administered estrogens), suggests a more direct influence of sex hormones.

The effect of a number of hormones on the retention of nitrogen and calcium was studied. The hyper- and hypothyroid states were both shown to be accompanied by diminished storages. In the hypothyroid, the administration of thyroid was followed by improved retention of calcium as a result of diminished stool losses. The effect on nitrogen, while essentially anabolic, was probably indirect and referable to increased intake and improved utilization of carbohydrate and fat. In one instance, an intake of calcium well above the normal requirement resulted in negative balances due to large fecal output until a depressed metabolism was corrected by thyroid.

The use of thyroid in small doses in the hypometabolic state resulting from prolonged inactivity was believed to be rational but, if practiced, an increased intake of nitrogen should be insured and the dose of thyroid kept small. One of its desired effects was an increased willingness of the patient to increase intake.

The effect of estrone administered to normal girls was catabolic, depressing the retention of calcium and nitrogen. When supplying a defect, estrone is considered to be anabolic. The assignment of any role in tuberculous reactivation to estrone would require the demonstration of the presence of excessive amounts of this hormone in the tuberculous adolescent. On this we have no information.

Focal infection not producing fever (chronically infected tonsils) was shown to depress retention even when intake was adequate. The removal of such a focus may in some instances be an important therapeutic measure in tuberculosis.

The effect of rest and activity was re-examined in tuberculosis. With elevated

temperature and pulse, the question does not arise. When these have become normal and, with prolonged inactivity, metabolism has fallen, the introduction of moderate amounts of activity tend to offset the undesirable effects of this state, so well demonstrated in disuse atrophy. In bed patients, pedalling a single wheel for one minute twice daily, and increasing by one minute, proved anabolic for nitrogen and calcium until an amount of exercise was reached which resulted in a rise in pulse and blood pressure, at which point retentions diminished.

The dietary requirement, with particular reference to calories, protein, calcium, and vitamin D, was re-examined. Caloric requirement seemed fulfilled by adding 70 per cent to the basal prediction from the developmental level on the Wetzel grid. This was not adequate in some advanced cases and it was necessary to increase intake without raising volume by concentrating foods with added amino acids or powdered milk preparations. When the caloric requirement was fulfilled, the nitrogen requirement was met if 15 per cent of the total calories derived from protein. We found that it was not possible to leave this caloric requirement to the chance of the child's selection, which tended to result in her taking less protein and relatively more carbohydrate and fat.

The calcium requirement of the girl whose growth had not ceased was found to be between 1.3 and 1.5 gm. In the period of rapid growth preceding menarche it was believed that she should receive 1,000 units of vitamin rather than the usually recommended 400 units.

*Metabolism studies on tuberculous adolescents:* There was a correlation between the adequate storage of nitrogen and a favorable course of the infection. Negative balances or inadequate retentions were associated with failure to heal, but it was not always possible to establish the fact that the negative balances were responsible for this failure.

This seemed to be true many times as judged by the time relationship between nitrogen loss and roentgenographic evidence of disease spread. The reverse situation undoubtedly obtains, namely, that a spreading process will result in a negative balance with intake unchanged, and that one effect of such measures as collapse therapy was to improve storage probably by diminishing "toxic absorption."

The disease process, even when unaccompanied by fever, would seem to be particularly toxic for nitrogenous tissue, as judged by the amounts required for positive balance and the extent of storage when adequate intake is maintained.

The problem posed by the tuberculous adolescent would seem to be chiefly the need for supplying at the same time amounts of food substances adequate for normal growth and the healing process and acquiring an understanding of the mechanism and of the offsetting of the diminished ability to retain nitrogen in the post-menarcheal period. That hormonal influences may operate through media other than their effect on the nitrogen metabolism is admitted; nor is the variability of the seed to be ignored. Massiveness and repetition of infection will remain unmeasured variables.

The adequacy of calcium retention would seem to bear some relationship to the preservation of the integrity of the primary lesion, but none to the healing

of the reinfection. In one instance a parenchymatous reinfection was noted at the site of a healed calcified tubercle following several months of failure to store normal amounts of calcium.

It is emphasized that many things other than the intake affect the storage of nitrogen and calcium. A broad consideration of all of these factors in an age when the growth process is particularly demanding in its requirements is essential to the proper management of the adolescent with tuberculosis.

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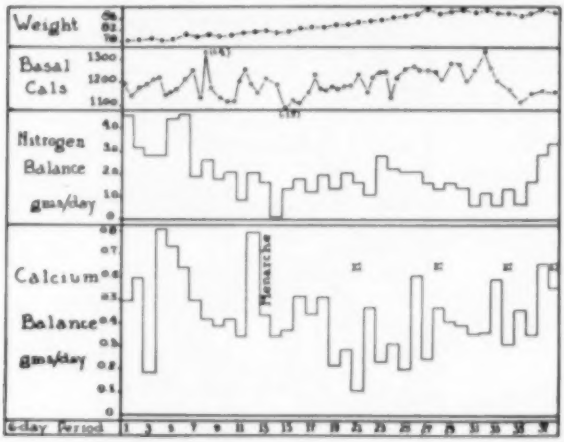


FIG. 1

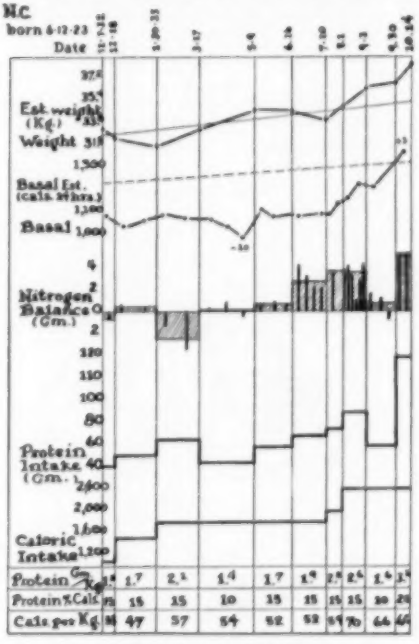


FIG. 2

Metabolism at Puberty

FIG. 1. (V. G.). Continuous nitrogen and calcium balances plotted in six-day periods for 228 days. The menarche occurred in the thirteenth period. Note the fluctuant character of the basal rate with a tendency to high points preceding and low ones following the menarche. With intake constant the pre-menarcheal retention averaged 2.69 gm. of nitrogen per day and 0.495 gm. of calcium per day; the post-menarcheal retentions averaged 1.57 gm. per day of nitrogen, and 0.387 gm. of calcium.

FIG. 2. (N. C.). Effect of suboptimal intakes: Weight may continue to rise with protein inadequate for normal retention. Part of the adjustment involves a depression of the basal metabolism. Increase in caloric and protein intake was followed by a rise in basic metabolic rate from minus 20 to plus 3 Boothby.

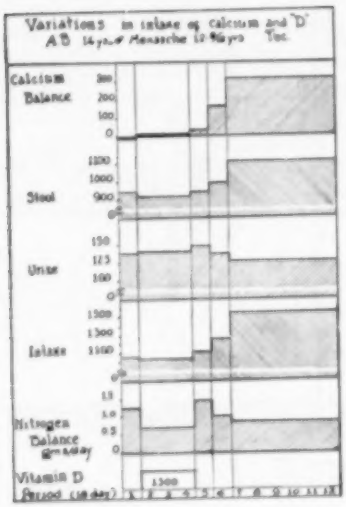


FIG. 3

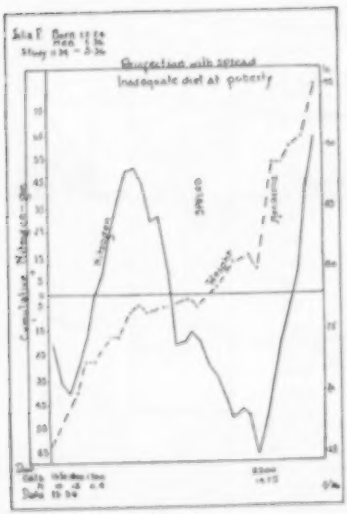


FIG. 4

Effect of Variations in Intake on Retention of Calcium

FIG. 3. (A. B.). No significant retention occurs here until the intake exceeds 1,100 mg. Vitamin D reduced fecal losses. Storage increases with increases in intake up to 1,500 mg.

FIG. 4. (J. F.). Following several months of nitrogen loss, the disease process spread. The failure of storage was referable to inadequate intake and probably the approach of menarche. Note the long period of loss of nitrogen while weight was being gained.

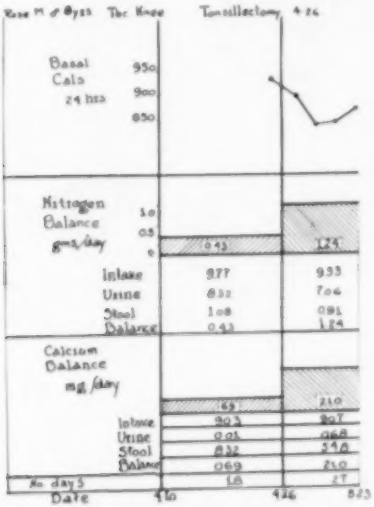


FIG. 5. (R. M.). *Effect of focal infection without fever on retention of nitrogen and calcium:* The removal of infected tonsils was followed by a threefold increase in the retention of nitrogen and calcium, the former referable to diminished urine output, the latter to diminished stool loss.

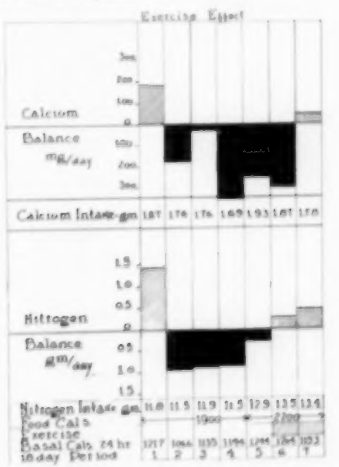
Exercise Effect
Calcium Balance mg/day
Calcium Intake gm 1.87 1.76 1.76 1.69 1.93 1.87 1.76
Nitrogen Balance gm/day
Nitrogen Intake gm 11.0 11.5 11.9 11.5 12.9 13.5 13.9
Food Calo 1900 2100
Exercise
Basal Calo 24 hr 1217 1066 1135 1196 1244 1264 1023
1 day Period 1 2 3 4 5 6 7

FIG. 6. (P. F.). *Effect of rest and activity on calcium and nitrogen metabolism:* A ten-year old girl was in normal balance while ambulatory in the ward as reflected in the first eighteen-day period of the observation. During the next seventy-two days at bed rest, with intake unchanged, balances were negative. Exercise in the form of pedalling a bed bike for periods up to nine minutes twice a day was followed by positive balances for both items.

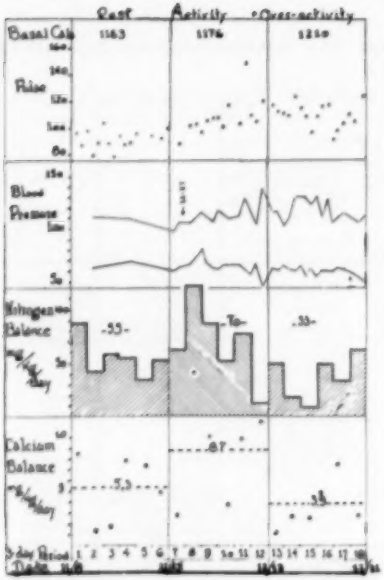


FIG. 7. *Effect of activity in pulmonary tuberculosis:* A 12-year old girl had been at bed rest for a year with a minimal type of reinfection tuberculosis when the exercise study was begun. While still horizontal, she pedalled a single-wheel bike for five minutes twice a day, increasing to thirteen minutes twice a day, at which time she showed fatigue.

Moderate exercise, short of fatigue, produced increased retentions of both nitrogen and calcium; both fell when the amount of exercise was sufficient to produce fatigue.

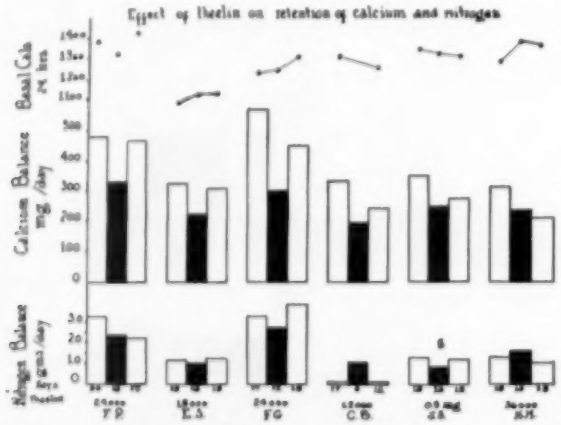


FIG. 8. Estrogenic substance (estrone) administered in amounts varying between 12,000 and 36,000 units to apparently normal girls resulted in a depression of the calcium balance, referable to increased fecal losses, the urinary excretion not being significantly changed. Nitrogen retention likewise fell in four of six subjects. Stilbestrol administered to one girl caused a diminution in the retention of calcium and nitrogen. The ages of the subjects were nine, ten, thirteen (three cases) and fifteen years. Two were in the pre-menarcheal, four in the post-menarcheal period.

This is at variance with what is reported in post-menopausal osteoporosis and might be understood in terms of the difference in response to a hormone when one corrects a deficit or, as might be the case with normal girls, creates an excess.

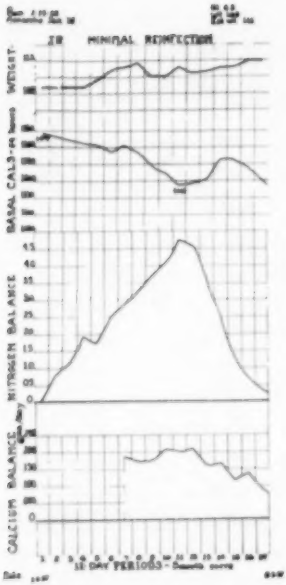


FIG. 9. (J. R.). *Regression of a minimal lesion at bed rest:* Steadily mounting retentions to a point which may represent a kind of "physiologic ceiling" followed by declining retentions in a fifteen-year-old girl two years past the menarche give a pattern characteristic of healing in a child not severely depleted and in a phase of growth close to termination. Retentions of as much as 4 gm. a day, however, do suggest a previous depletion.

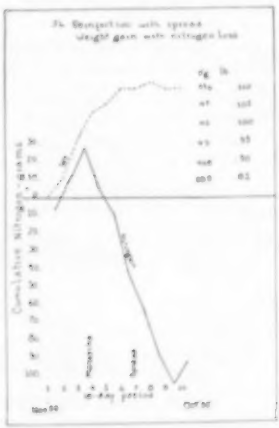

FIG. 10. (J. L.). A girl was admitted with a minimal reinfection type of lesion and was being studied to determine nutritional requirement of this age. In the first forty-five days there was a steady gain in weight but a positive nitrogen balance only with high intakes of nitrogen. The negative balance at the menarche could be attributed to a diminished intake but, immediately following the menarche, the balances were negative on amounts (13.2 gm.) which before the menarche gave positive balances. At this time a frank spread of the tuberculous lesion was detected which required collapse therapy. Note that weight continues to be gained while nitrogen is being lost.

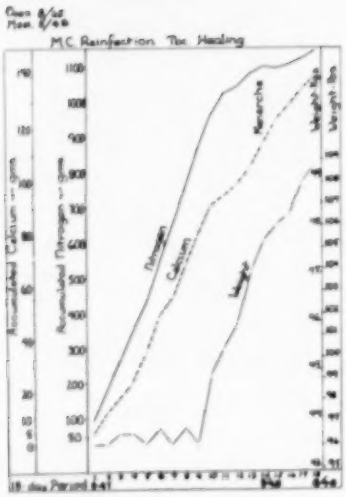


FIG. 11. (M. C.). The reconstitution of tissue when a change is made from low protein-high carbohydrate diet to an adequate protein diet. This latter derived its calories as follows: 15 per cent from protein, 35 per cent from fat, and 50 per cent from carbohydrate. The weight curve was flat for 162 days, during which time nitrogen and calcium depleted stores were replenished. For the next 162 days, gain in weight was normal and was accompanied by normal retentions of nitrogen and calcium.

Before the study was begun and a fixed diet prescribed, there had been an increase in the extent of the tuberculous lesion; regression was rapid from the beginning of the study.

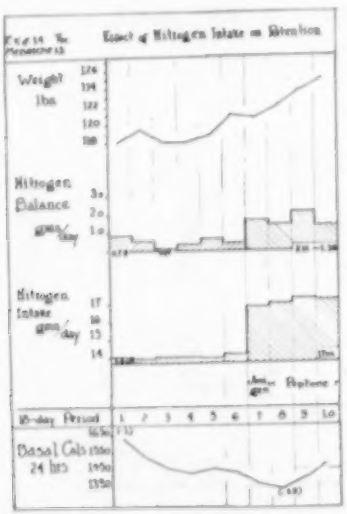


FIG. 12. (Z. K.). *Effect of added amino acids on retention:* In the presence of inadequate storage and refusal of food, raising the intake with added amino acids would seem logical. The addition of 3 gm. to the intake resulted in an increase in retention. A similar result was achieved by concentration with powdered milk.

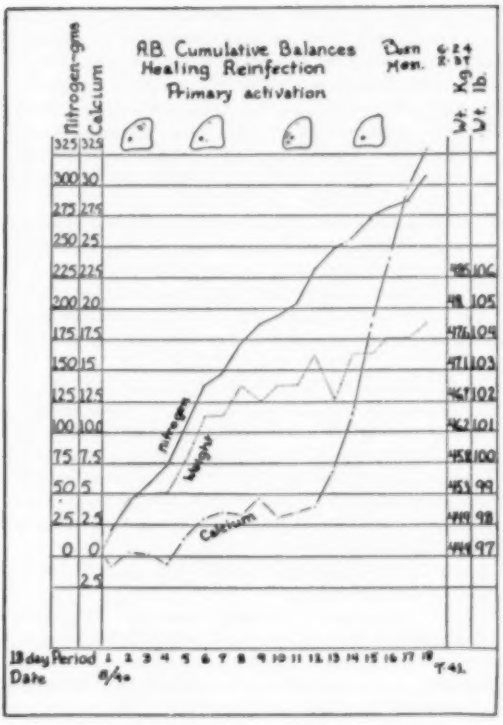


FIG. 13. (A. B.). *Possible relation of failure of calcium retention to reactivation of primary:* Nitrogen balance was positive throughout a period when an upper lobe reinfection was healing with pneumothorax therapy. Accumulative calcium balance for the first seventy-two days was −579 mg. Shortly after this a fresh reinfection was evident at the site of the calcified Ghon tubercle in the right middle lobe.

(All of the foregoing charts are reprinted from *Nutritional Studies in Adolescent Girls and Their Relation to Tuberculosis* by Joseph A. Johnston and are reprinted by permission of Charles C Thomas, Springfield, Illinois.)

## GENERAL DISCUSSION

DR. HILL: I think Dr. Johnston has placed before us at least one explanation for the reason why children and adolescents seem to have a recrudescence of their tuberculous infection. The subject is open for discussion.

DR. MIDDLEBROOK: Just to start the discussion, I want to say that Dr. Johnston apologized, on first coming here, about presenting data and material that he feared might have no pertinence today. I tried immediately to disabuse him of this. I would like to introduce the discussion in that fashion. Dr. McDermott, would you care to comment on these studies?

DR. MCDERMOTT: As Dr. Johnston is the first to know, this whole problem of the background of nutrition in tuberculosis is extremely difficult to find a handle to. In his very carefully documented studies he has found a couple of handles.

I have become particularly interested, as several of us have, in sudden changes in body state with relation to the development of tuberculosis or to its spread. I was particularly interested in the slide he showed of the young girl who was losing nitrogen all summer long, but gaining weight all the time, and then suddenly something happened.

Apparently, in so far as either the development or the spread of tuberculosis is concerned, it is what happens *suddenly* that counts. If one loses weight suddenly, that's bad. Or, as Dr. Holmes in Seattle is showing with his "skid row bums," it is equally as bad to suddenly stop drinking as it is to suddenly start drinking. It is the suddenness that counts.

I think the study of the patient with the nitrogen fall-off took us behind the scenes a bit, to show that these things which appear suddenly are really not so sudden when one has such meticulous observations as were made by Dr. Johnston.

DR. ALEXANDER: Dr. Johnston has himself raised the question of whether or not the exacerbation or reinfection of tuberculosis can or could be responsible for the nitrogen balance, or whether the negative nitrogen balance caused by other factors is responsible for reinfection.

That leads me to ask whether the negative nitrogen balance could be ascribed to poor appetite or poor economic status, and, therefore, to poor protein intake.

DR. KASSOWITZ: I was very much interested in the relation between nitrogen storage, calcium storage, proper nutrition, and immunity or resistance to infection.

We introduced a different study on the adequacy or inadequacy of a supposedly ideal nutrition for tuberculous children at our sanatorium. On paper it was ideal: an adequate diet, 15 to 20 per cent protein, with adequate caloric contents. That was on paper. But then I looked at the meal schedule.

Breakfast at 7:30 a.m. to 7:45 a.m. Supper at 4:30 p.m. to 4:45 p.m. That leaves a fasting interval of fifteen to sixteen hours at night.

We then studied blood sugar levels, realizing that the carbohydrate is the least important, yet is one index of adequate nutrition. The fasting blood sugar level after fifteen or sixteen hours was very low, if not actually hypoglycemic.

Then I introduced an 8 p.m. lunch with 15 to 20 per cent protein; and, lo and behold! the fasting blood sugar level after eleven and one-half hours was very much higher.

This drives home Dr. Johnston's point that nutrition on paper is not actual nutrition. I think we should consider, especially with the institutional diet which is figured out beautifully on paper by the dietician and checked or not checked by the doctor in charge, that, if one looks into it, one will find that shortening the twelve-hour fasting interval will also help the nitrogen-calcium storage. This may happen as a result of supplying a proper total intake of a proper amount of food instead of the fifteen- to sixteen-hour starvation period.

DR. MIDDLEBROOK: May I suggest that Dr. Johnston be given an opportunity to answer the questions directly? I think it is a little more stimulating to do it that way than by storing up a pile of questions.

DR. JOHNSTON: If I may answer Dr. Alexander first: Of course, that was the obvious problem and I did try to develop the business of whether this is cause or effect because a spreading lesion will pretty consistently be accompanied by a negative balance. The only thing I would emphasize is the timing.

The negative balance obviously precedes the spread that we could demonstrate by X-ray. Again, true, you can have quite a bit of inflammation before it is demonstrable on the roentgenogram; so you have to seek other things for support.

Second, the evidence that there was a previous depletion, as judged by the magnitude of the balances—and that would take a year, as we did, constantly getting back and forth a correlation between spread and negative balance, and between healing and a positive balance; the crux of that would always be timing.

Why they went into negative balance is another long story which is developed at length in the book I wrote. Usually it was just poverty. These children were all from the lowest economic stratum, and from those countries where the protein intake is habitually low.

One of these patients, for example, was from Italy, and it took me three months to persuade her to take any protein at all without nausea. She seemed not to have had it before.

To go on to the next problem: we did a separate study to see the extent to which, left to themselves, the children would select a good diet, somewhat like what Clara Davis did on a young child years ago. Only one of four voluntarily would select 15 per cent protein. They preferred the carbohydrate. A long indoctrination in a home where the carbohydrate intake was the usual thing, I think, probably created a pattern that was pretty hard to break. So, by merely offering protein, we didn't get anywhere.

Your point about the interval of fasting is a very good one, Dr. Kassowitz. Just as I came away, I had to throw out the balance record of a patient because we found that he had been cheating. I don't mind their cheating if I know what they have, but I can't add up the intake without knowing that. He said, "I just get too hungry at night, that's all." In his case it wasn't a problem of underfeeding. He got it all right, but it wrecked my balance studies on him for the last month.

The evening lunch is a much better way. We do it both with rheumatic fever patients and with tuberculous patients, particularly in hospitals and sanatoriums where things seem to be geared with a little too much of an eye on the convenience of the people who are going to serve and wash the dishes.

I think regular provision needs to be made for a mid-afternoon snack, too. At Saranac the evening snack at 9:30 p.m. probably ran about 500 calories, with milk and eggs. It was a process of stuffing. The four- or five-meal plan definitely works out better.

DR. KOCH-WESER: Concerning the anabolic effect and the catabolic effect, would not one suggest some difference in the natural history of tuberculosis during puberty?

DR. JOHNSTON: As you know, the rise in rate of active tuberculosis consistently occurs two years sooner in the girl than in the boy, if I get the point you are making. It is true that testosterone is highly anabolic for nitrogen, although reciprocally catabolic for calcium. I don't know whether that makes much difference.

Concerning the estrogen thing, Dr. Lurie, of course, feels that he can demonstrate what might be a beneficial effect with the estrogens, but probably through the medium of its influence on the spreading phenomenon. I still would like to see some correlation between the nitrogen story and, say, hyaluronidase, but I have not done anything like that except in rheumatic fever.

DR. WILLIAM MANDEL (Denver, Colorado): I would like to ask whether some of these biochemical changes can be used to increase disease activity.

DR. JOHNSTON: I am sure they could. It would take a little too long to go into all the side alleys we have gone down and the green fields we have gone through. We have done studies with cortisone and ACTH, but not in connection with tuberculosis. I wanted to, but I was talked out of that years ago. Anyhow, in rheumatic fever the initial exhibition of either ACTH or cortisone, as you know, is followed by a veritable outpouring of nitrogen that lasts from twelve to sixteen days. Then the effect on appetite asserts itself, and a terrific increase in intake quickly offsets that; so you have the stress reaction, and a stress reaction of any degree would unload as much as 3 gm. a day, a truly negative balance. I have observed that in some rheumatic fever patients. However, as I say, that is quickly offset. It also has the advantage of completely depressing the basal metabolic rate, a desirable thing in a child with an active rheumatic carditis, and of dropping the pulse and temperature.

DR. MITCHELL: Dr. Johnston, do you know how much nitrogen loss can occur from a heavy production of sputum and from diarrhea in patients with intestinal tuberculosis?

DR. JOHNSTON: I don't know about the sputum. I do know from menstruation. It is negligible. You can forget it. The whole week's period would not contribute more than 0.5 gm. From the stools it can be terrific. Its effect will be doubled. Not only will the fecal losses increase, but the nitrogen losses as shown through the kidney will reflect a toxic state.

Those studies have been done more in infancy than in other age groups, and intestinal

manifestations of tuberculosis in this age group have been practically negligible. I have had only two wards, although for a long time I had four wards, of tuberculous children.

DR. DAVID RADNER (Chicago, Illinois): We have now started to document a series of cases in which we have been following well-healed, arrested, or inactive cases of tuberculosis in adults, but we have a series now in which, in one instance within eight days—and in most of them within ten or twelve days—an explosive pneumonic tuberculosis occurs. We have films eight or ten days apart showing the healed lesion at first and the massive fulminating tuberculosis occurring after severe enteritis. The origin of the enteritis has been bacterial in most cases.

DR. JOHNSTON: I like to think, in a preventive sort of way, that maybe our figures reflect something that Dr. Lincoln was just telling me, that is, the improvement one might expect from a program designed to do something about this.

Dr. Lincoln tells me that now, in their follow-up, 10 per cent of their primaries have developed reinfection-type tuberculosis. In twenty-five years, in our series, only 3 per cent have. I think the advantage accruing to these children by becoming orphans has been their transfer to a good country home where they have been able to get decent nutrition, whereas her group has still been compelled to live in underprivileged circumstances in the city.

I was a little naive about the advantages of farm life, however. I just took it for granted that, with all those cows, everything was going to be taken care of. I was amazed to find the one thing that they were hesitant to give the children was milk because that had been sold in advance. They could have beef and eggs and cheese, but the milk was a cash crop with which one bought clothing. They changed that policy after we discovered it.

DR. GRZYBOWSKI: I wonder if I may be allowed a rather general and perhaps naive comment on this subject that Dr. Johnston has been talking about.

He focused our attention on the point that chronic pulmonary tuberculosis always or practically always occurs at puberty. When I was in medical school the answer seemed simple. We read that the child got infected, went to school, led a protected life, and reached the teens, when he went out and got reinfected; and there was a sort of Koch's phenomenon in his lung, and there was the reinfection tuberculosis.

Now we see that, in addition to those children whom we follow who seem to be prone to develop reinfection tuberculosis—always around adolescence or in the early twenties—in addition to those people, nurses who happen to have been infected at seventeen or eighteen may develop the same condition of chronic pulmonary tuberculosis within a few weeks or a few months after primary infection.

I do feel that these theories (and there are quite a number of them) which were introduced by the great investigators early in the century were wrong. We must agree that they were wrong. Those great men uncovered a great many facts, and they will always be remembered for those facts. However, some of their theories were wrong.

I also believe that certain nomenclatures that they introduced, and which we have inherited, may occasionally inhibit our own efforts in finding the answers. I am speaking of terms like "endogenous" and "endogenous reinfection," "superinfection," "reinfection tuberculosis." I do not want to suggest that those terms are meaningless, but maybe sometimes we give them more meaning than they really deserve; and sometimes we use a word instead of thinking.

I feel we all ought to be grateful to Dr. Johnston for his studies on the reason why chronic pulmonary tuberculosis often occurs at this age. I do feel that, if our investigations followed his footsteps, and if more work were done to answer the simple question of why it is, maybe we would get another powerful weapon in our fight against tuberculosis.

DR. WALDO E. NELSON (Philadelphia, Pa.): I would like to support Dr. Grzybowski's statement on nomenclature. It is perhaps a minor note. The term "endogenous reinfection" is not a good one and does not say what we want it to say. The term used by Dr. Terplan yesterday, "exacerbation," would seem to be preferable, or simply "activation of a quiescent lesion," if you will, or "reactivation," but not "endogenous reinfection."

DR. TERPLAN: I would prefer to leave the discussion as it is. This is too important. I

tried to clarify the nomenclature in a monograph fifteen years ago, and I am surprised that these statements have been made. I think it is very important today to realize that a clear nomenclature may be very essential. The time of the first infection has changed, and those of us who have already had some opportunity to examine first infection lesions in the young adult, where some of the immunologic collateral evidence is available—at least as far as disease activity is concerned—have learned that quite a few of the classic phenomena of the typical primary infection are changing or have changed.

I want to stress that in about 20 per cent of all of the resected specimens which I have examined, usually in lobectomies, I have had the opportunity to prove that we are dealing with what appeared as the so-called adult or reinfection type of tuberculosis, and that it all could be very nicely correlated with a first infection gradually spreading, without, however, producing extensive lymph node lesions in this 20 per cent. The lymph node lesions were minimal in contrast to the massive inflammation in the classic primary lesion at the same time after infection.

I think that the more carefully these things are analyzed, and the more we stick perhaps to what we try to designate as true primary, really truly exogenous, infection, the less confusion we will have in the future. It will be very important now, because, as the pathologist sees it, the picture has changed very much.

**Dinner Meeting**

Thursday Evening, November 10, 1955

# CHANGING ATTITUDES TOWARD THE THERAPY OF TUBERCULOSIS[1]

SIDNEY H. DRESSLER[2]

This Symposium has been largely concerned with morbid organic processes and with the effects of potent chemotherapeutic substances on the course of tuberculosis in the pediatric age group. It might be wise to inquire into the profound psychological consequences that specific antimicrobial therapy is having on adults afflicted with this disease because the adult constitutes the great reservoir for childhood tuberculosis in this country, and the eradication of the disease in children stands or falls on acceptance or rejection of treatment by adults.

Tuberculosis, it has been said, serves the unconscious needs of some patients so well that infection with the germ is less a stroke of fate than a kind of pact with the devil. While there does not appear to be any consistent pattern of personality disturbance in tuberculosis, such as in peptic ulcer and ulcerative colitis, it may be said very generally that the tuberculous have problems with dependency. Sometimes there is conflict of dependency drives versus drives for independence; sometimes, more or less frank surrender to dependency strivings; or there is actually reactive hostility, consequent upon the thwarting of these needs.

Psychiatrists point out that dependency conflicts are well nigh universal in our culture. Regardless of the pre-morbid situation and the basic personality structure of the patient, the treatment of tuberculosis before chemotherapy exaggerated dependency strivings with its insistence on long-term bed rest and limitation of physical activity even after stabilization of disease. Who cannot remember the days of such aphorisms as: "If you feel like running, walk; if you feel like walking, sit down; and, if you feel like sitting, lie down."

There were those to whom this philosophy was eminently acceptable, and it is not surprising that these patients elected to refrain from any type of productive activity thereafter, becoming, with a few outstanding exceptions, a drain upon their families, communities, and society in general.

There were those to whom this philosophy was completely unacceptable. These actively rebelled, refusing treatment, leaving sanatoriums, and adding to the reservoir of infection in the community.

Today, dependency-encouraging factors in treatment can be enormously reduced, provided both medical personnel and patients are willing to give them

[1] Address delivered at the Symposium dinner held at the National Jewish Hospital on the evening of November 10, 1955.

[2] The National Jewish Hospital at Denver, and the University of Colorado School of Medicine, Denver, Colorado.

up. Most of us in this hall would agree that the most effective use of chemotherapy involves giving the medicaments in adequate dosage, consistently and uninterruptedly, over extended periods of time. Not all of us would agree, I fear, to diminished use of bed rest or its actual abandonment. Suffice it to state here that experimental and clinical evidence is accumulating that immobilization is perhaps not a necessary adjunct in treatment. The burgeoning employment of ambulatory regimens and the closure of institutions designed to offer little more than good nutrition, fresh air, and bed rest testify to the increasing acceptance of this latter concept by the profession at large.

A popular song of the day has it that, "It Takes Two to Tango," and I believe this title aptly describes the situation existing today between medical staff and patient: on the one hand, the patient clinging desperately to his disease or rebelliously acting out his hostility by forgetting to take his medication or leaving against medical advice; on the other hand, staff, many of whom are ex-patients, tending to infantilize or needlessly restricting patients, and in various ways reacting blindly in terms of their own needs.

If patients are to be effectively motivated to continue chemotherapy for protracted courses whether in hospital or on home-care programs, then enlightened rehabilitation must be practiced with emphasis on consideration of the emotional factors involved, and occasionally even long-term psychotherapy will be needed. Staff must be helped to minimize their own projections, to handle their own dependency needs and reactive hostility, if they are to see each patient in a realistically helpful light.

Although acceptance of specific therapy is a primary goal in the control of tuberculous disease, it is not the only one. In a broader sense modern medicine strives to return to society an individual free from organic disease, capable of assuming personal, family, and community responsibilities. A concept such as this implies that the individual will be physically able to work—that he will have been prepared for some vocation. Not every patient, of course, will need to acquire a new occupation. There are many, however, who never have had a vocation and will have to be trained in one compatible with physical status and aptitude.

Thus far we have been considering the tuberculous individual with the favorable prognosis: the one who responds to chemotherapy, with or without surgery, with sputum conversion and inactivation of disease. Fortunately these comprise the vast majority of all new cases if the patients *stick* with treatment. There is a residue, however, of sputum-positive cases, mostly in the middle and older age ranges, which are beyond the help that modern therapy can offer. Specific therapy has had the effect of prolonging life without inactivating disease in these patients.

It is estimated that there are about 150,000 open cases of tuberculosis in our population not known to the authorities. These unregistered or "whereabouts unknown" cases are likely to be those who have had bitter hospital experiences or those who are the sole support of their families, or—what it is the fashion to call today—the recalcitrant patient.

The persons in the environment of the known case can at least be protected

from having tuberculous infection flare into tuberculous disease. The persons in the environment of the "recalcitrant" are at his mercy. Forcible hospitalization programs are being advanced as a solution to this problem. I respectfully suggest that, if any of the points made in this presentation so far have any validity, then a program designed to treat, educate, and rehabilitate the patient offers a far better chance to eradicate tuberculosis than incarceration.

Recent federal legislation encourages the formation of sheltered workshops. The open custodial case of tuberculosis can find a haven here. Opportunity to earn, the feeling of being able to contribute to society, the acceptance rather than the rejection by one's fellow men would see the ranks of the recalcitrant shrink.

If I were asked to express an opinion on what has been the greatest effect of specific therapy on attitudes toward tuberculosis, I think it is the development of a positive philosophy of treatment—a philosophy which recognizes that the fight should be carried to the tubercle bacillus instead of appeasing the foe by avoiding measures which might irritate him into renewed aggression. The patient need no longer be repeatedly urged to submit supinely to his disease and accept the status of second-class citizen. Our treatment need no longer convert the potentially productive individual into the passive, fear-ridden professional invalid. The ex-tuberculosis patient can be and should be a contributing member of society.

## Session 5

Friday Morning, November 11, 1955

J. Arthur Myers, *Honorary Chairman*

Sidney H. Dressler, *Moderator*

# SYSTEMATIC TREATMENT OF PRIMARY TUBERCULOSIS

Robert Debré[1]

In this paper I should like to consider in a general way the problem of the treatment of primary tuberculosis in infancy and childhood.

Three points must be stressed: first, the importance of early diagnosis. We now possess many very effective drugs against tubercle bacilli, and we know that these drugs must act early. A late diagnosis of acute tuberculosis, either miliary or meningeal, entails dramatic consequences. Second, it should not be forgotten that the onset of subacute or chronic tuberculosis is usually fairly sudden and obvious, and treatment should be begun at this time.

Third and last, in our opinion, we must emphasize the necessity for an early discovery of patients with symptom-free onset, only known by the recent appearance of a positive skin reaction to tuberculin. We think that these cases also should be treated immediately.

As we know, even symptom-free tuberculous infection in children results in the development of one or several primary nodules with large regional adenitis and, together with the formation of these lesions, in a dissemination which sometimes may precede the establishment of allergy.

A. Monbrun and Lavat found choroid tuberculomas in 6 per cent of patients with primary tuberculosis, a definite evidence of hematogenous dissemination. The future of the child depends on the activity of the primary pulmonary and lymphatic foci and on diffuse unapparent and minimal lesions. The treatment of the recently infected child is mandatory if we want to prevent acute generalized tuberculosis and secondary bone and other lesions, and the early and late pulmonary manifestations in adolescence and adult life.

Moreover, we know that the action of chemotherapy is very strong on recent and early developing lesions. The drugs inhibit the growth of large bacterial populations, eventually killing them and altering the characters of the remaining organisms (drug sensitivity, pathogenicity). These organisms, still alive in the lesions, probably result in the persistence of allergy and of a real immunity.

The action of chemical agents cannot be separated from the natural reactions of the infected host. Therefore, the question arises as to the optimum time to begin the treatment—a question imperfectly solved so far, despite experiments in men and animals, either (*a*) in the ante-allergic or pre-allergic stage, (*b*) in the hyperergic stage, or (*c*) in the following allergic stage. In our opinion, and

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without conclusive evidence, drug therapy should begin during the hyperergic stage, characterized by strongly positive allergy and sometimes by fever, erythema nodosum, and associated phenomena.

The treatment is not designed to control the clinical manifestations, but to stop active lesions and to prevent the further occurrence, early or late, of tuberculous manifestations.

Therefore, now more than ever, we must be vigilant in our detection of the penetration of the tubercle bacillus into the child. The routine use of tuberculin tests in children, the repetition of these tests, their performance immediately in children whose health deteriorates without reason are mandatory. The use of this excellent method of mass preventive medicine and of individual testing of infants, adolescents, and young adults should not be diminished.

We propose to report some data regarding both the risk run by the recently infected child and the advantages of the routine, early treatment of all cases of primary tuberculosis.

Frolich's statistics are some of the most accurate of the first ones to be recorded. They cover 1,830 school-age children of Oslo, Norway, who had a positive tuberculin test at the age of seven years in 1912. In 1925, at the age of twenty years, 87.8 per cent seemed untouched by tuberculous manifestations. This means that between the age of five and twenty years, 12.2 per cent displayed tuberculous manifestations, involving the lungs (4.5 per cent); the pleura (3.9 per cent); the bones, joints and lymph nodes (3.4 per cent); and the meninges (0.38 per cent).

Later, S. N. Meyer found comparable figures in a group of 2,146 children followed from 1929 to 1944. Heimbeck noted a rate of 0.5 per cent of pulmonary tuberculosis during the first year after contamination in children less than twelve years old, whereas Malmros and Hedwall, Hyge and Gedde Dahl found a figure of 10 per cent in adolescents. Ustvedt reported 3 per cent of pleurisy and 1 per cent of extrathoracic tuberculosis shortly after primary infection.

In the United States, Tasi and Dasche, in a survey of 600 cases, tabulated (shortly after tuberculous invasion) miliary tuberculosis in 1.2 per cent of the cases, meningitis in 2.4 per cent, bone tuberculosis in 3.2 per cent, and a pulmonary cavity in 4 per cent. Miller followed up 258 children contaminated before the age of five years; during the first two years of observation he saw 16 cases of miliary or meningeal tuberculosis (4.1 per cent). Thirty-six children (3.5 per cent) died of tuberculosis. The figures reported by Dr. Lincoln are even higher.

The French statistics established in collaboration with Brissaud and by A. Lotte provide similar results. The risk of early tuberculous meningitis, as related to the number of contaminations, is 3.38 per cent; that of chest tuberculosis is 4.5 per cent. Grossly and schematically, of 1,000 school-age children contaminated at the age of seven years, on the average, more than 120 present a tuberculous localization before the age of twenty, including pleurisy in 30, pulmonary involvement in 45, and tuberculous meningitis in more than 3. In addition, their fate is unknown after the age of twenty years.

The danger in childhood is not the same for all. The basic elements of prognosis should be kept in mind.

* * *

The child's age is a factor of major importance, for the severity of tuberculosis in very young children is well known. In children less than three years old, morbidity for extrapulmonary tuberculosis only rises to 5 to 10 per cent with a fatality rate of 3 per cent, due to the high incidence of meningitis in that group. The risk is greater in infants less than one year old. Some years ago, with L. Bernard, we found mortality rates (mortality, *not* morbidity) of 24.25 per cent in the age groups zero to six months and 5 to 6 per cent in the age groups six to eighteen months. These figures have changed but little.

Tuberculosis is also especially severe after the age of thirteen years in girls and after fifteen years in boys. Morbidity with pulmonary tuberculosis, which is 0.3 per cent yearly up to the age of thirteen years, rises suddenly. If primary tuberculosis occurs after the age of fifteen, the yearly incidence reaches 2 and 3 per cent during the three or four following years, thus realizing a total morbidity rate of more than 10 per cent.

Another factor also accounts for the severity of the disease—the importance of contact. Holmdahl found a familial contamination in 13.4 per cent of cases of common, uncomplicated primary tuberculosis, whereas he found it in 24.7 per cent of cases of complicated primary tuberculosis.

As to tuberculous meningitis, familial contagion was noted with a very significant frequency. In 418 cases of acute tuberculosis, including 293 of meningitis, we found that the child had been contaminated by the father or the mother in 34 per cent.

Many years ago we had shown with L. Bernard that the severity of primary tuberculosis in the infant was strictly related to the intimacy of the contact and to the amount of bacilli released by the infecting subject. We had tried to establish laws of prognosis of primary tuberculosis on the basis of age of the child and the importance and the duration of contact.

We always contrasted familial contamination and "street tuberculosis," as we named it, due to random contamination.

Can we find elements of prognostic value in the very features of primary tuberculosis? Ustvedt and Johannesen found in children who were affected with erythema nodosum in the course of primary tuberculosis a slightly higher morbidity than in others. This fact was not found by Meyer. In Meyer's opinion, a high erythrocyte sedimentation rate, during the first quarter following the infection, is correlated with a lower morbidity due to pulmonary tuberculosis in subjects more than eighteen years old. The finding of bacilli in the gastric contents gives the opposite prognosis.

Various authors agree more readily on the value of the X-ray findings. Holmdahl saw the development of pulmonary tuberculosis twice as frequently when he had seen a roentgenographic primary focus. This observation was made in a statistically valid number of children. It seems that these manifestations may be

considered as aggravating factors, but there is no significant difference between children who do and those who do not display these manifestations.

In children ranging from three to thirteen years of age, the over-all prognosis is good. Nevertheless, the lack of clinical or roentgenographic manifestations does not permit an absolutely favorable prognosis.

To sum up this consideration of prognosis, it is possible to recognize the importance of certain factors, but it is not possible to conclude, if these factors are lacking, that the course of primary tuberculosis will be favorable. Although the risk is less in favorable circumstances, it is not negligible and, above all, can never be foreseen.

On the basis of these considerations, French doctors consider it necessary to treat actively all infants. This includes children at the pre- and post-puberal stage; those with marked primary tuberculosis, with fever, accelerated erythrocyte sedimentation rate, obvious X-ray findings, erythema nodosum, and those with evidence of a massive contamination.

But the difficult problem is the management of school-age children with recent, symptom-free tuberculosis but positive tuberculin reactions. Under these conditions, treatment may be useless or, at least, superfluous, since primary tuberculosis is a curable infection, conferring immunity. On the basis of the experience gained with streptomycin, we know that long-term treatment of primary tuberculosis cannot always prevent accidents of the primary infection or the further development of other tuberculous localizations. Shall we see an identical situation with isoniazid, with persistence of lesions containing isoniazid-resistant bacilli?

Although these reservations are sensible, the uncertainty of prognosis, the therapeutic value of isoniazid, the low risk of bacterial resistance support the case for routine treatment. Certainly, we must give it a trial instead of merely speculating on the matter.

Therefore, to clarify the problem, the French National Institute of Hygiene, under the direction of Professor L. Bugnard, with the help of the Social Security Organization, is carrying out a study of the question. Some thousands of children, all treated since the discovery of primary tuberculosis, and the same number of control children (treated only with rest, fresh air, and "climatic cure") will be investigated.

This inquiry is not finished at present, and no definitive conclusions have been reached so far. Nevertheless, two investigators, Drs. Raynaud and Naveau, have observed the following facts: In cases with normal tomographic roentgenograms of the lungs and mediastinum, usually no pathologic changes occur under treatment. When primary tuberculosis is accompanied by opacities in the lungs, either unique or multiple (very likely due to tuberculous lesions), they constantly and rapidly disappear under the drug treatment. On the contrary, without treatment they persist for a long time in most cases. Atelectatic opacities are not markedly modified by the treatment. The same is true for very enlarged lymph nodes and lymph node-bronchial fistulae.

As to the bacteriologic course, we occasionally have found acid-fast bacilli on direct examination of the gastric contents. The latest cultures were those of the fifth month after the beginning of the treatment; the strains were susceptible to PAS and to isoniazid. In contrast to the untreated cases, not a single case of pleurisy has been seen in children treated more than eighteen months.

As to tuberculous meningitis, the relatively small number of treated subjects and the relative rarity of the disease do not permit a statistically valuable conclusion.

However, in 2 cases an interesting finding was made: A meningeal involvement, most likely of tuberculous origin, developed under treatment. The children had been treated for two months and four months, respectively. Without modifying the treatment, both recovered. The time of onset of these meningeal incidents suggests that they were not the spontaneously curable, allergic meningeal reactions which occur during the hyperergic stage of the first month of treatment. After that period of time, we see true tuberculous meningitis, which usually does not respond to further treatment.

* * *

*In conclusion:* On the basis of eighteen months of study of about 3,000 children and controls, it seems that treatment promotes excellent response of lung opacities due to tuberculous disease. It is less effective on adenitis and atelectatic opacities. However, the results are not negligible; and the longer the time elapsed since the onset and its treatment, the greater the differences between the treated children and the controls. We would stress again that we are not reporting conclusions, but preliminary findings.

Routine treatment was carried out as follows: We consider streptomycin a valuable weapon to be kept in reserve against the possible occurrence of a severe accident. Therefore, we do not use it. Isoniazid is easily ingested, diffusible, and of low toxicity for the children; it is given in a dosage of 10 mg. per kg. of body weight, without exceeding a total dose of 400 mg. in two divided daily doses. Dosage is doubled in infants less than two years of age.

To avoid resistance, PAS is also given in a dosage of 300 mg. per kg. of body weight per day, in one daily dose. Contrasting with the administration in the adult, PAS is easily taken by children and well tolerated by the digestive tract. Treatment must be uninterrupted. A prolonged treatment is of major value.

All experiments in men and animals demonstrate the possibility of obviating re-treatment, which is often necessary when treatment is stopped too early. We should be energetic enough to continue the treatment over six to eight months, and in no case for less than six months. No noticeable side effects have been seen. Only in cases of acute disseminated tuberculosis did we observe the occurrence of fever with rash or of purpura.

Apart from this comparative study, designed to give information on the value of the routine treatment of the infected child, to be assessed only after several years, we shall speak of some special indications in the course of complicated primary tuberculosis. First, cortisone should be used in the treatment of severe

forms with high and protracted fever, of primary and post-primary serofibrinous pleurisy, and of distressing respiratory disorders related to large mediastinal adenopathies. Aspiration bronchoscopy is also useful in the treatment of sudden attacks of dyspnea as a result of lymph node-bronchus fistulization. In such cases surgical excision is also helpful. As these topics are very controversial, I mention them only at the end of this paper.

# EFFECTS OF ISONIAZID ON ALLERGY

## With Some Additional Observations in Children Vaccinated with BCG

C. CHOREMIS,[1] D. LIACACOS,[1] I. ALLAMANIS,[1] D. ZOUMBOULAKIS,[1] AND H. KOURTI[1]

Previous observations, in our clinic as well as by other investigators (specifically on the problem of tuberculous meningitis), emphasize the injurious effects of tuberculin hypersensitivity which are exerted on the course, treatment, and prevention of the complications of tuberculosis (1–7). The use of the new chemotherapeutic agents in combination with tuberculin and cortisone for the treatment of childhood tuberculosis places the problem of allergy, its relation to immunity, and its significance for the development of tuberculous infection on a broad basis.

The therapeutic differences between streptomycin and isoniazid are, according to our opinion, mainly differences in the action of each drug on the allergic state of the host organism. Our investigations indicate that streptomycin fortifies rather than weakens allergy (8). We therefore, concentrated our interest on the study of the effect of isoniazid on the allergy of tuberculosis.

It is the purpose of this paper to present our observations on the effect of isoniazid on the clinical, biologic, and histologic phenomena which develop during the course of a tuberculous infection, artificially created by BCG vaccination.

We have divided our study into the following four parts:

1. Influence of isoniazid on BCG vaccination in general
2. Influence of isoniazid on Koch's phenomenon
3. Influence of the isoniazid-cortisone combination on BCG vaccination
4. Influence of PAS on BCG vaccination

### INFLUENCE OF ISONIAZID ON BCG VACCINATION IN GENERAL

*Material and methods:* Our material consisted initially of 60 children, one to fifteen years old, who reacted negatively to an intradermal injection of 5 units of PPD tuberculin eight days before BCG vaccination.

These children were vaccinated with 0.1 ml. of BCG in the region of the left deltoid muscle. In a few of them we observed the characteristic scar of a previous BCG vaccination, which, however, was not taken into consideration in the classification of the children into the four groups described below. During the experiment we lost contact with 13 of them. Our study is based, therefore, only on observation of 47 patients.

We randomly divided these children into four groups:

*Group A:* 10 children. The administration of isoniazid was started four days before vaccination.

*Group B:* 14 children. Isoniazid administration was started simultaneously with vaccination.

[1] From the Pediatric Clinic of the University of Athens, Athens, Greece.

*Group C:* 10 children. Isoniazid was administered beginning one week after vaccination.

*Group D:* 13 children, who were left without isoniazid therapy and were, therefore, used as controls. In the first three groups isoniazid was given in a daily dosage of 4 to 6 mg. per kg. of body weight.

*Clinical course:* The vaccination was well tolerated by all of our patients. No side effects were noted. From the perusal of the literature available to us, we could not find anything related to the effect of isoniazid on the macroscopic lesions which develop after a BCG vaccination.

We followed the local redness and infiltration initially every second day after vaccination and subsequently at weekly intervals. Both were measured in millimeters. (The vaccine was supplied by the Pasteur Institute of Athens.) These observations covered a period of approximately eleven weeks.

The comparative determination of the mean diameter of redness and infiltration of the children of each group was made by obtaining a mean value of measurements on each child and for each group of children.

Our results on children vaccinated for the first time were as follows:

*Group A:* Average redness 2.5 mm.; infiltration 1.8 mm.
*Group B:* Average redness 3.3 mm.; infiltration 2.4 mm.
*Group C:* Average redness 3.7 mm.; infiltration 2.9 mm.
*Group D:* Average redness 5.9 mm.; infiltration 4.8 mm.

On patients previously vaccinated, we observed a slight phenomenon of Koch, which consisted in a redness, 8 to 20 mm. in diameter, and an infiltration of 6 to 15 mm. which appeared within the first forty-eight hours after vaccination. This proved the existence in these children of an allergy which could not be elicited by Mantoux test with 5 units of PPD.

The Koch phenomenon was not as pronounced in patients who received isoniazid. The average redness and infiltration in patients who received isoniazid (4 children in *Group A* and one patient in *Group B*) were 8.2 and 5.7 mm., respectively, while the control *Group D* (not receiving isoniazid) had values of 10.4 and 8.3 mm. It is evident from our results that the inhibitory effect of isoniazid was slightly more pronounced on the infiltration than on the redness.

In none of the children vaccinated for the first time and receiving isoniazid (*Groups A*, *B*, and *C*) did local suppuration take place. In those who did not receive isoniazid, suppuration occurred in 9 of 11 patients (81.8 per cent), in the fifth to seventh post-vaccination week. In all previously vaccinated children, suppuration was observed whether isoniazid was administered or not.

Regional adenitis appeared in patients vaccinated for the first time as well as for the second time who, as already mentioned, developed a mild Koch phenomenon.

The course of the adenitis, more pronounced in second vaccinations, especially in patients who did not receive isoniazid, was as follows: From the first post-vaccination day, a lymph node enlargement (one or more) to the size of a bean or nut occurred. In no case did suppuration of the nodes occur.

From the above it is concluded that isoniazid exerts an inhibitory effect on local reactions. The sooner the administration, the greater was the effect. The inhibitory action of isoniazid on local suppuration was also noted in all children receiving the drug, while up to 81.8 per cent of patients without isoniazid medication suffered a suppuration.

In partially allergic children isoniazid had no apparent effect on suppuration. This probably is due to the fact that the usually occurring bacterial dissemination does not take place in these patients, and the tubercle bacilli remain for a long time, alive or dead, at the site of vaccination. We will further analyze this point.

Besides the inhibitory action of isoniazid on the local reaction, it appears that the drug had a similar effect on the regional adenitis, which occurs after a BCG vaccination.

*Biologic course. Testing of allergy:* There is no general agreement on the effect of isoniazid on allergy (9–14). We studied allergy both with PPD and with the BCG vaccine material itself.

The testing of allergy with PPD was evaluated one week after vaccination and at weekly intervals thereafter. A total of nine such intracutaneous tests with 5 units was done on each child. Two weeks after the last injection and 11 weeks after vaccination, a Mantoux test, using 10 units of PPD, was done. The testing of allergy with BCG was done by the method of Delachaux and Bergier (15). This seems to be a more delicate method of evaluating the allergic state than is the tuberculin skin test.

The results on children vaccinated for the first time, using PPD (10 units), were as follows:

*Group A:* All of the 6 patients reacted negatively.
*Group B:* Twelve of 13 children reacted negatively.
*Group C:* Eight of 10 patients had a negative reaction.
The total of patients from these three groups who reacted negatively was 26 of 29 (89.6 per cent).
*Group D:* All 13 children, regardless of whether they were previously vaccinated or not, showed a positive reaction.

Of 5 children vaccinated for the second time (4 in the first and one in the second group), only 2 showed a negative reaction.

It was considered important to investigate whether the absence of allergy in all of these patients was permanent or lasted only while isoniazid was being administered. It was likewise important to evaluate whether isoniazid would have any effect on patients who were tuberculin positive. Accordingly, in 6 of the children who were receiving isoniazid and who showed a negative tuberculin reaction, the administration of the same dose of isoniazid was continued for one additional month, while in 6 similar patients the isoniazid administration was stopped. Furthermore, in 8 additional children who had been vaccinated for the first time and had not received isoniazid (*Group D*-tuberculin positive), the drug was administered for one month.

The results have shown that the test done at the end of the month's observation with 10 units of PPD was again negative in the previously tuberculin-

negative children, whether isoniazid was continued or not, while 3 of the 8 tuberculin-positive patients became tuberculin negative.

The BCG test was applied only in the above 20 children. It was always positive in tuberculin-positive children while it was negative in all tuberculin-negative children with the exception of 2 of 3 above-mentioned tuberculin-positive children who had become tuberculin negative. These two children reacted positively to the BCG test.

Our conclusions from the effect of isoniazid on allergy are the following:

BCG vaccination, accompanied and followed by isoniazid administration for a period of eleven weeks, resulted in the inhibition of allergy in 89.6 per cent of children vaccinated for the first time, and in 40 per cent of children vaccinated for the second time.

Allergy was still absent one month after stopping isoniazid treatment.

Isoniazid administered for one month to patients who had recently become allergic as a result of BCG vaccination produced a negative tuberculin reaction in 3 of 8 children.

The BCG test appears to be superior to the tuberculin reaction when at least 10 units of PPD are used.

*Appearance and agglutination titer of antibodies:* It is well known that a BCG vaccination is followed by the appearance of antibodies in the serum (16–19). It was decided to investigate whether isoniazid would have any effect on the time of appearance as well as on the titer of these antibodies. The blood of 2 children randomly taken from each group (total of 8) was examined before the BCG vaccination for the presence of antibodies, according to the method of Middlebrook-Dubos. The examination was repeated one week after vaccination and at different intervals thereafter. The agglutination test became positive in all children with titers up to 1:8 except in one child in whom the titer was 1:4.

It should be mentioned that the first child who, one week after vaccination, had already developed a titer of 1:8, was the only one who was previously BCG vaccinated. The results thus show that isoniazid does not seem to have an

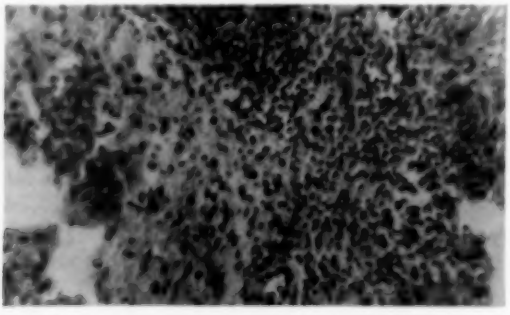

FIG. 1. Section of skin lesion from *Case 31633* (no isoniazid), showing a diffuse infiltration of the dermis by acute and chronic inflammatory cells. Caseation, tubercle and giant cell formation are absent.

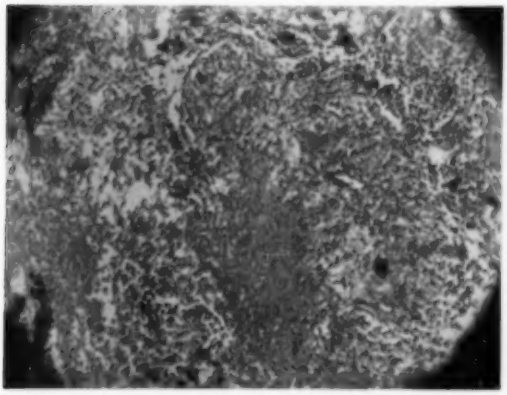

FIG. 2. Section of skin lesion from *Case 31635* (also no isoniazid), showing typical changes of tuberculosis with caseation, tubercle formation, and few Langhans' giant cells.

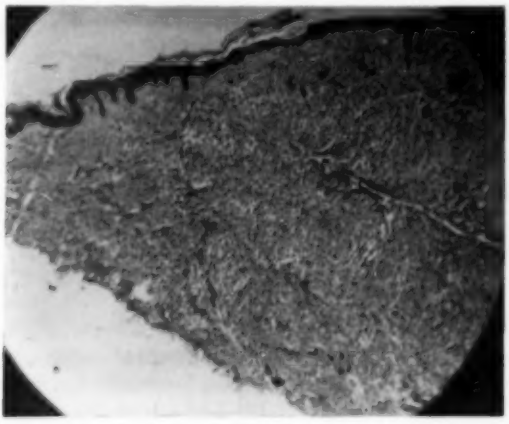

FIG. 3. Section of skin lesion from *Case 31634* (isoniazid-treated) showing the circumscribed infiltrate. Note the well-demarcated lesion and the zone of normal corium beyond the infiltrate.

effect on antibodies, at least as far as their time of appearance and the titer of agglutination is concerned.

*Electrophoretic studies of the serum:* Through the literature available to us, we could not find, with the exception of a fatal BCG infection (20), whether or not BCG vaccination produces any electrophoretic changes of the serum. We had observed previously that a violent reaction after a BCG vaccination may produce changes similar to those seen in active tuberculosis (21). Accordingly, an attempt was made to test whether an uncomplicated BCG vaccination produces any electrophoretic changes or whether isoniazid would have any effect

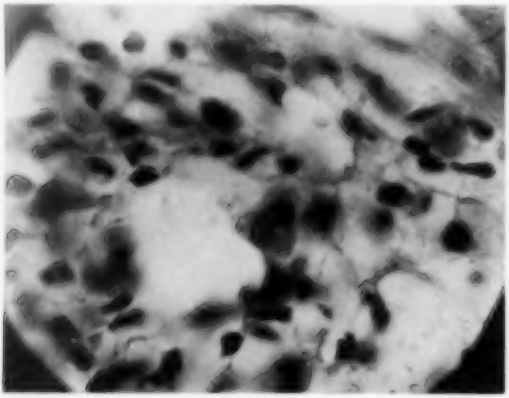

FIG. 4. High magnification of the same lesion shown in figure 3. Note the fibroblastic proliferation and the abundant fibrillar ground substance.

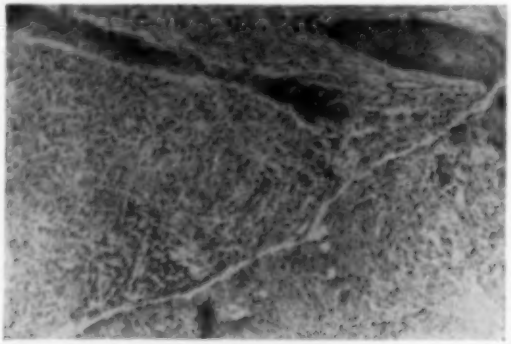

FIG. 5. Higher magnification of the lesion from a patient treated with isoniazid (*Case 31632*), showing the various cell types (fibroblastic, histiocytic, and epithelioid-like). The cell with the large, lobulated, lightly stained nucleus and the abundant eosinophilic, granular, and slightly foamy cytoplasm corresponds to the so-called "reversed" epithelioid-macrophage cell described by Dick. Such cells were scattered within the infiltrate of both patients treated with isoniazid.

on such changes. Specimens of serum from the above-mentioned 8 children whose Middlebrook-Dubos reactions had been determined were studied with electrophoresis. The results revealed no changes of the protein fractions of the serum, whether or not the patients were receiving isoniazid.

*Histologic studies:* The effect of isoniazid on the histologic picture of tuberculosis has already been investigated (22, 23). However, it was decided to study the effect of isoniazid on the histologic picture of BCG-vaccinated children at nine weeks after vaccination. The entire infiltrated area of the vaccination in 2 children of *Group A* and 2 of *Group D* was excised. The tissues were examined

histologically and studied for the presence of acid-fast bacteria. It should be mentioned that the 2 children from *Group A* revealed, for the group, the strongest local reaction, while the 2 from *Group D* were the only ones who did not show local suppuration (figures 1–5).

No acid-fast bacteria were found. From the histologic examination, the impression was formed that the lesions in patients treated with isoniazid were more circumscribed; showed no caseation or typical tubercles, a less inflammatory reaction, and a more pronounced fibroblastic proliferation. The observed macrophages were of interest and might have represented the so-called "reversed" epithelioid-to-macrophage cells described by Dick (22).

### INFLUENCE OF ISONIAZID ON KOCH PHENOMENON

Twenty-two children, aged six to twelve years, were vaccinated with 0.1 ml. of BCG in the region of the left deltoid muscle. They all reacted negatively to 10 units of PPD four days before vaccination. They were then divided into two groups. The first included 11 children to whom isoniazid (4 to 6 mg. per kg. of body weight daily) was administered on the same day with vaccination and was continued for a period of seven weeks. The second group included 11 children who were to serve as controls without isoniazid.

Four weeks after vaccination all children were revaccinated with the same amount and brand of vaccine over the right deltoid muscle. A Mantoux test with 10 units of PPD was simultaneously done. A second Mantoux test with 10 units of PPD was done one week after the first, and was again repeated for a third time (four weeks after the first and three weeks after the second Mantoux). The observations were continued for a period of nine weeks. It should be mentioned that, according to our experience from the use of our strain of BCG, we consider the Koch phenomenon to be positive whenever a local infiltration of 5 mm. or more develops within the first week.

*Clinical course:* Both the first and second vaccinations were well tolerated with the exception of the vaccination of one child of *Group B* who developed a temperature of 99.5° to 101.3°F. of three days' duration, starting two days after the second vaccination.

The average diameters of the areas of redness and infiltration on the first and second vaccinations were:

*Group A:* First BCG average redness 3.4 mm.; infiltration 2.9 mm.
Second BCG average redness 5.3 mm.; infiltration 3.9 mm.
*Group B:* First BCG average redness 5.6 mm.; infiltration 4.8 mm.
Second BCG average redness 13.9 mm.; infiltration 11.4 mm.

In the children of *Group A* (isoniazid-treated), infiltrations at the site of the second vaccination varied at the end of the first week from 3 to 12 mm. in diameter, while in 6 children (54.5 per cent) they were 5 mm. or less. In the children of *Group B* (no isoniazid), infiltrations of the second vaccination ranged in size from 12 to 22 mm. at the same time. These results were interpreted as confirming the previous observations that isoniazid had a stronger inhibitory effect on infiltration.

The incidence of suppuration was as follows:

*Group A:* First BCG—in none did suppuration occur (0 per cent).
Second BCG—suppuration occurred in one patient (9.1 per cent).
*Group B:* First BCG—suppuration occurred in 10 children (90.9 per cent).
Second BCG—suppuration occurred in 10 children (90.9 per cent).

Regional adenitis was comparatively weaker than at the first vaccination and developed even less in both vaccinations if isoniazid was administered. There was no apparent influence of the second vaccination at the site of the first vaccination.

*Biologic course:* The studies of the biologic course were limited to the application of the above-mentioned three tuberculin reactions in order to follow the course of allergy in each child. In *Group A* (isoniazid-treated), three consecutive Mantoux reactions were negative with the exception of that of one child who showed a positive result only on the third Mantoux test.

In *Group B*, the first Mantoux reaction was positive in 8 patients; the second Mantoux reaction was positive in one patient; and the third Mantoux reaction was positive in 10 patients.

Thus, the effect of isoniazid on local lesions, and particularly the redness and infiltration of second vaccinations, was again demonstrated. It entirely prevented the appearance of the Koch phenomenon in 6 of 11 children, while the Koch phenomenon in the remaining children was weak.

As far as allergy is concerned, it was inhibited in 10 of the 11 children. The Mantoux test (10 PPD units) done ten months after the second vaccination in 7 of these 10 children was again negative in 5 and weakly positive in the remaining 2 children. The temporary disappearance of allergy at the second Mantoux test in *Group B* was due to the second vaccination.

### INFLUENCE OF THE ISONIAZID-CORTISONE COMBINATION ON BCG VACCINATION

In 4 children who reacted negatively to 10 PPD units, isoniazid (4 to 6 mg. per kg. per day) and cortisone (2 mg. per kg. per day) were administered simultaneously with BCG vaccination. The isoniazid-cortisone administration was continued for a period of five weeks in 2 children and seven weeks in the other 2 children, when excision and histologic examination of the local lesion were done in all 4 cases. The combination of these two drugs resulted in such a strong inhibitory action on local reactions at the site of vaccination, as well as on the regional adenitis, that, occasionally, the site of inoculation became almost unidentifiable, particularly during the first month.

Repeated Mantoux tests were negative until the third month after vaccination when observations were stopped.

Histologic and antibody studies in these patients are in the process of being performed.

### INFLUENCE OF PARA-AMINOSALICYLIC ACID (PAS) ON BCG VACCINATION

The administration of para-aminosalicylic acid (PAS) did not significantly alter the development of the primary complex, either from the clinical or from

the biologic point of view, in 8 patients (150 mg. per kg. of body weight daily) as compared with 4 controls (not receiving PAS).

## DISCUSSION

From the above, the anti-allergic effect of isoniazid on tuberculosis, which is apparently potentiated by the combination with cortisone, is well documented.[2] The anti-allergic properties of isoniazid are more pronounced in subjects in whom allergy has recently developed.

The fact that suppuration is inevitable whenever allergy exists (the suppuration proves the *in situ* action of the etiologic agent) is in favor of a local reaction's being related to the extent of allergy. The higher the allergy, the more potent and prompt is the expected reaction (experimental phenomenon of Koch).

This phenomenon is reversed whenever isoniazid is administered, i.e., the drug's action is more potent in cases in which allergy has recently developed. Thus, cortisone's inhibition of the development of allergy, on the one hand, promotes the multiplication and dissemination of the *Mycobacterium tuberculosis*; on the other hand, it enhances the bactericidal action of isoniazid.

Precisely in this respect isoniazid is superior to streptomycin which, according to our investigations, not only lacks anti-allergic properties but possibly increases allergy (8). This has been shown by the fact that, in newborn babies infected by their parents, streptomycin administered during the pre-allergic state, in contrast to isoniazid (14), cannot prevent allergic manifestations (25).

If allergy has been acquired in the past, isoniazid exerts a quantitative limitation of it, but cannot prevent a local suppuration. This is probably explained by the fact that the bacteria responsible for allergy have been organized and the drug can only partially reach them.

Suppuration is probably due to pre-existing allergy which later disappears as a result of the action of isoniazid.

Of interest also is the observation that the development of antibodies (tested by the reaction of Middlebrook-Dubos) is not affected by the administration of isoniazid. Thus, allergy and circulating antibodies seem to be two different functions of the host. The theoretical and practical significance of this observation is obvious. Changes of electrophoretic patterns of serum proteins have not been observed. It has been shown, however, in previous studies in our clinic that the agglutination titer of the Middlebrook-Dubos reaction and electrophoretic changes have a parallel course, while the tuberculin reaction develops independently (18, 21).

Allergy has always been considered of paramount importance in the course of tuberculous infection. Clinical and experimental evidence supports that observation. As has already been mentioned, the introduction of new chemotherapeutic agents has placed the whole problem on a new basis by modifying the

[2] Studies *in vitro* in our clinic on the effect of cortisone on the development of *Mycobacterium tuberculosis* do not support the view that the drug inhibits the growth of the microorganism (24).

clinical and biologic picture of tuberculous infection. Of specific interest in this matter has been the study of tuberculous meningitis. We consider it the model disease in which allergy plays a major role in the manifestations and clinical course and which can be followed and, to a certain extent, controlled.

The significance of allergy for the interpretation of the clinical picture of tuberculous meningitis had already been emphasized in the past.

Wallgren (26) has shown that tuberculous meningitis is a continuation of the primary complex. The later observation, that during this phase the *Mycobacterium tuberculosis* circulates in both the blood and the cerebrospinal fluid, as our studies have shown (27–28), has further elucidated and clarified the pathogenesis of the disease and the role of allergy.

This early bacterial phase, which should be considered as a continuation of the pre-allergic one (29), requires a revision of the anatomic concept of the primary complex, which should be considered in a more dynamic sense. Worth mentioning is the fact that bacteremia and invasion of the cerebrospinal fluid which appear in cases of primary tuberculosis continue during the clinical manifestations of the disease and are the last to disappear after the treatment of tuberculous meningitis (30).

It is not easy to define the factors which, besides the presence of the tubercle bacilli, contribute to the development of tuberculous meningitis. The role of allergy should not be ignored.

Clinical and biologic observations support this view and define, up to a certain point, the mode of action of different therapeutic agents, as well as the difference of actions of streptomycin, isoniazid, cortisone, and tuberculin. These observations, we believe, are further reinforced by the above experimental evidence. The inhibition of the appearance of the phenomenon of Koch, for which the absence of allergy should be considered responsible, is also an indication that, in the absence of hypersensitivity, violent hyperergic reactions (in which we include tuberculous meningitis) can hardly be produced.

To this anti-allergic property of isoniazid (besides its direct action on *Mycobacterium tuberculosis*) we attribute not only its therapeutic superiority in tuberculosis in general, but also its particular prophylactic effect against tuberculous meningitis.

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# CORTISONE AND CORTICOTROPIN IN THE TREATMENT OF TUBERCULOSIS IN INFANCY AND CHILDHOOD

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The question of the use of cortisone during the course of infectious diseases is of great interest and importance at present.

In spite of the fact that the early results of the use of cortisone alone in infectious processes (especially tuberculosis) were unfavorable, experimental and clinical trials of cortisone combined with chemotherapy were encouraging. The experimental results have been conflicting, however, and this can be explained to a certain extent. Nevertheless, a great deal of work remains to be done to clarify this important problem.

Experimentally, it was demonstrated early that animals naturally resistant to tuberculosis develop a lethal infection when they are treated with cortisone and, at the same time, are infected with tubercle bacilli (1). Other investigators have confirmed these findings. They have reported that, in the experimental tuberculosis of guinea pigs or mice, the animals treated with cortisone demonstrated a greater spread of the disease and an increased number of organisms present in the various involved organs than were present in the controls (2–5). Other investigators, on the contrary, had reported that the use of cortisone or corticotropin resulted in a less serious course of the disease in the experimental tuberculosis of guinea pigs. Still others reported that guinea pigs infected with tubercle bacilli and treated with cortisone revealed no significant variation from the controls (6, 7).

In agreement with the last cited authors, we have shown that cortisone alone does not lead to significant changes in the course of experimental tuberculosis in guinea pigs. Histopathologic studies by Ragazzini (8) revealed that, although there were no appreciable differences between control and cortisone-treated animals, the caseous necrosis clearly evident in the controls was less extensive in the animals treated with cortisone. In animals subjected to the action of cortisone, proliferation of epithelioid cells appears to be more marked. Infected guinea pigs treated with streptomycin alone also show an enhanced production of epithelioid and giant cells, but in this case there is no evidence of caseous necrosis.

On the other hand, cortisone combined with streptomycin produces a significant improvement in the course of the disease in infected guinea pigs, greater than that found with streptomycin alone. This indicates that cortisone enhances the chemotherapeutic action *in vivo* of the antimicrobial against tubercle bacilli. Guinea pigs treated with cortisone in combination with streptomycin show a decrease in the number of nodular lesions. These are not typically arranged in micronodular formation, as occurs in guinea pigs treated with streptomycin alone, nor is there evidence of necrotic foci.

On the whole, infected guinea pigs treated either with streptomycin alone or with streptomycin and cortisone demonstrated that the disease process tends

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to be limited by important productive and localizing phenomena. Limited, that is, in the case of the administration of streptomycin alone and in the case of combined streptomycin-cortisone treatment, by a widespread productive reaction and less typical nodular lesions which probably result from an inhibiting action on the specific granuloma which is demonstrated by cortisone.

The explanation for the different results obtained by various investigators is that different dosages were employed. In laboratory investigation of the physiologic and physiopathologic activity of cortisone, the doses of cortisone and corticotropin used are generally much higher than those used therapeutically in human beings.

In rats, guinea pigs, and rabbits, doses of several milligrams were used, and these correspond to 10 to 20 mg. per kg. in human beings. These doses naturally gave very different biologic responses. Streptomycin was employed in large doses in experimental tuberculosis, and this certainly modified the evolution of the disease. In fact, some studies carried out by Chemi (9) indicated that large doses of streptomycin, injected into guinea pigs during inoculation with diphtheria antitoxin, resulted in inhibition of passive immunity. It is possible that guinea pigs treated with excessive doses of streptomycin likewise may have the mechanisms of defense similarly inhibited. Ragazzini used relatively small doses of streptomycin (15 mg. per kg. in guinea pigs) as well as cortisone (about 6 mg. per kg.).

Corticosteroids act not only on lymphoid tissue, as shown by lymphocytolysis of thymus cortex (10), but they also stimulate the phagocytic activity of the macrophages. It may be that such an action enhances defense mechanisms against bacilli. Some authors have demonstrated that phagocytosis by the macrophages is enhanced by the action of cortisone, which produces marked storage of bacilli within these cells. This storage may reflect a decreased digestive capacity of these cells for bacteria.

The different results obtained by various investigators in their studies of the effect of cortisone on infections (especially tuberculosis) are certainly due to the following elementary principles used in the technique of the experiments:

Different results may be obtained if different doses of cortisone are used. Totally different results are obtained when streptomycin is administered with cortisone. It would appear that excessive doses of streptomycin might inhibit the mechanisms of resistance.

The response to the use of cortisone depends on the size of the infecting dose of bacteria. In the presence of a massive infection, every defense mechanism fails. These are not the conditions observed in naturally acquired tuberculosis. A small infecting dose reproduces phenomena more similar to those seen in clinical tuberculosis and makes possible a more strict evaluation of the data.

The time at which cortisone treatment is begun in infected guinea pigs is of great importance. Cortisone will display, of course, a different action if its administration precedes, is simultaneous with, or follows the beginning of the infection.

In human beings, cortisone alone should be used only a long time after the primary infection. Knowledge of the degree of hypersensitivity of the patient is of great importance. The more intense the allergic reaction to tuberculin, the more effective will be the action of cortisone, e.g., in erythema nodosum and in perifocal allergic reactions.

Cortisone in infectious diseases (especially tuberculosis) must always be administered under antimicrobial "protection." The important conclusions of studies on the action of cortisone alone in clinical material, as well as in the tuberculosis of guinea pigs, lead us to believe that in human beings it is absolutely necessary to use antimicrobials whenever cortisone is to be used.

The available clinical evidence indicates that antimicrobials and cortisone improve the defenses of human beings against infection, especially against tuberculosis. Here the tissues are rich in local defense mechanisms which in turn tend to help the organisms defend themselves against the antimicrobials. In clinical tuberculosis, antimicrobials apparently are hindered in their action against those organisms which are enclosed in the tubercle.

It should be mentioned that the allergic factor plays an important role in human tuberculosis, and that this allergy is influenced by cortisone. Corticosteroids certainly attenuate the clinical and histopathologic manifestations of tuberculin hypersensitivity. Our studies have shown that the intradermal tuberculin reaction under the influence of physiologic doses of hydrocortisone is significantly diminished by comparison with controls tested with the same dose of tuberculin. However, it is necessary that a diluted solution of tuberculin be used (1:1,000 to 1:5,000 to 1:10,000) (11).

* * *

These experimental data recently have been applied clinically. We have studied the effect of cortisone and corticotropin, especially in the treatment of tuberculous meningitis. The profound action of cortisone in infectious and allergic processes suggested its use in order to help solve certain serious problems that would have been difficult to overcome in any other way. Cortisone proved to be very effective apparently in preventing the appearance of obstruction to the circulation of cerebrospinal fluid. Moreover, cortisone apparently removed obstructions to the flow of cerebrospinal fluid, which might otherwise have led to serious degrees of hydrocephalus. Cortisone, with high antiphlogistic, antifibrinous, and antifibroblastic power, injected either systemically or (better still) locally, may prevent or remove exudates, allowing the free flow of cerebrospinal fluid and better diffusion of antimicrobials everywhere.

The addition of cortisone to the treatment of tuberculous meningitis was considered in 1952 by Prosperi and Rossi (12). Our initial data are based on the results of treatment of 30 patients. Prosperi and Rossi showed that cortisone administered intramuscularly or orally in the dose of 25 to 50 mg. daily (in some cases hydrocortisone was injected intrathecally, 2 to 10 mg. per day) had a favorable effect in the treatment of both adults and children with tuberculous meningitis. There was a noted decrease in the incidence of obstruction to the flow of cerebrospinal fluid. The protein content uniformly decreased rapidly. The disease ran a shorter course and sequelae were rare. The death rate was reduced (in these 30 cases the death rate was 10 per cent). It should be emphasized that cortisone was administered only in the more serious cases in which a block was thought to be present. At the same time, Esente (13) employed cortisone or hydrocortisone, both systemically and locally (by the cisternal route), in 29

cases of amblyopia which developed during the course of tuberculous meningitis. Amblyopia due to chiasmatic arachnoiditis showed satisfactory improvement. Peripheral vision significantly improved, while central vision showed a less favorable change. Satisfactory results have been obtained as well in the amblyopia following hydrocephalus due to meningitis.

More recently Cocchi (14) has begun hydrocortisone therapy with intracisternal administration in patients affected by tuberculous meningitis and has obtained striking results. Cortisone or hydrocortisone treatment is combined with that chemotherapeutic regimen which has been used at our institution for the treatment of tuberculous meningitis.

The regimen is as follows:

(*a*) *Intramuscular and intravenous:* Streptomycin (dihydrostreptomycin), 300 to 500 mg. per day by intravenous drip the first week or so; thereafter the same doses intramuscularly. Isoniazid, 300 to 900 mg. per day, orally or intravenously.

(*b*) *Lumbar intrathecal injection:* Streptomycin (sulphate or calcium chloride salts—not dihydrostreptomycin), 20 to 30 mg. per day, and isoniazid, 20 to 40 mg. per day.

(*c*) *Intracisternal injection:* Isoniazid, 20 mg. per day plus hydrocortisone, 2.5 to 5 mg. per day, alternating (*b*) and (*c*) every twelve hours or using two cisternal injections (*c*) daily at twelve-hour intervals. Streptomycin is never used intracisternally.

In some cases hydrocortisone was also injected by the lumbar route, but the results were almost the same as those obtained with intracisternal treatment. Final results indicated that the cisternal route gave much better results than the lumbar one for injection either of isoniazid or of hydrocortisone.

Mori (15) studied the "diffusion index" of hydrocortisone injected by cisternal and lumbar routes at doses of 2 to 10 mg. He demonstrated that hydrocortisone injected into the meningeal space diffused from the lumbar segment to the cephalic one, and vice versa. By lumbar injection, diffusion was slow and not complete, requiring more than eight hours for stabilization of lumbar and cisternal levels.

Cisternal injection resulted in diffusion into the lumbar segment more rapidly, and higher hydrocortisone levels were found after four to eight hours in the lumbar cerebrospinal fluid by comparison with those of the cisternal cerebrospinal fluid.

In the 15 patients so treated (adults and children), no instance of intolerance or of pain was reported. A marked and consistent decrease in the protein content occurred.

The satisfactory and rapid resolution of tuberculous meningitis treated with hydrocortisone injected by cisternal route, combined with antimicrobial therapy, is apparently due to the greater diffusibility of hydrocortisone injected intracisternally.

The duration of treatment with hydrocortisone is usually two to four weeks. When the cerebrospinal fluid protein has returned to normal values, ordinary systemic treatment with injection of antimicrobial by the lumbar route may well be sufficient.

In some cases, interruption of hydrocortisone treatment has led to an increase

in the protein content of the lumbar or the cisternal cerebrospinal fluid. Then re-establishment of initial treatment for a few days has returned the protein levels to normal.

It is very important to treat patients with tuberculous meningitis (or with other systemic forms of tuberculosis) with the most complete and specific antimicrobial therapy available, combined with corticosteroids.

When the clinical course and bacteriologic studies tended to indicate to us that the tubercle bacilli were resistant to the action of streptomycin we also employed viomycin sulfate, which has an action similar to that of streptomycin.

Viomycin has been extensively studied by Masi and Giusti (16), Masi and Moggi (17), Masi and Dettori (18), Giusti and Barilli (19), and most recently by Giusti (20). These studies showed that viomycin (15 to 20 mg. per kg. per day) is very effective and that it does not cause serious toxic phenomena. The few neurotoxic lesions caused by viomycin are generally reversible, in contrast to the neurotoxic lesions of streptomycin.

* * *

The course of tuberculous meningitis has been studied in more than 1,000 cases. We have excluded from our total statistics only those patients who were transferred from other hospitals. These patients were suffering from chronic obstruction and productive disease, apparently the result of inadequate treatment. This differs entirely from tuberculous meningitis which has been treated with adequate therapy from the start. This form of the disease deriving from inadequate treatment can still be treated even when the condition of the patient is grave and the patient is in a state of coma.

Review of our clinical cases makes it clear that, as techniques have improved, there has been gradual progress. In 1947 there was systemic therapy plus lumbar spinal injection, using streptomycin and a sulfone. In 1948 we began using intracisternal therapy and para-aminosalicylic acid systemically. In 1952 isoniazid was added. At this time intraventricular treatment was more frequent and, later on, antimicrobial treatment using intravenous drip was added. In response to each technical improvement, the recovery rate increased even though there were some fluctuations which were undoubtedly the result of the more frequent appearances of strains of resistant bacteria.

The introduction of cortisone and especially of hydrocortisone in intracisternal therapy has resulted in a notable increase in the percentage of total recovery. This increase has reached 97 per cent in the last 38 cases, whereas the general curve of ordinary treatment without cortisone has taken a downward trend.

* * *

I wish to conclude this paper with a few remarks on the possibility of healing tuberculous meningitis without local treatment, employing only systemic therapy with streptomycin and isoniazid in high doses. I believe that prolonged observation of the many cases we have treated, coupled with unsparing analysis, reveals that it is possible to heal some cases of tuberculous meningitis with systemic treatment alone in mild cases and especially in the cases in which treatment is started early. It is certain, however, that some cases require both systemic

therapy and local therapy (lumbar, cisternal and ventricular puncture, if necessary) and that this double approach results in the highest recovery rate. We have seen that this rate approaches 97 per cent. It is, therefore, contraindicated to employ a method or scheme of therapy simply because it is somewhat less difficult to carry out.

I wish to add a few words about the treatment of various tuberculous lesions in childhood combining cortisone with antimicrobials. The indications for the use of cortisone and corticotropin are slowly being extended. Recently, cortisone preparations have been used in tuberculosis in childhood. We believe that this drug may be an important addition to chemotherapy. Ragazzini has studied the data on a group of tuberculous patients, in which the tuberculin reaction, as well as clinical evidence, gave clear proof of a state of hypersensitiveness which dominated the general picture. It was logical to think that in this particular state corticotropin and cortisone could improve the results of treatment by diminishing the intensity of the allergic reaction.

Six cases of exudative pleurisy have been treated with the introduction into the pleural cavity of hydrocortone (25 to 50 mg.) one to four times every two to three days combined with streptomycin (150 to 200 mg.) and isoniazid (20 to 40 mg.). Cortisone in the dose of 1 mg. per kg. per day was also administered orally, or a similar preparation was employed in slightly smaller doses. Of course, standard systemic therapy with intramuscular streptomycin injections (10 to 20 mg. per kg. per day) and isoniazid orally (10 to 15 mg. per kg. per day) was carried out in every case.

The clinical results have been excellent and in some cases even dramatic. It is especially the rapidity with which the exudate disappears and the fall in temperature occurs, as well as the marked improvement in general condition, which is so characteristic of these patients. In addition, the tuberculin hypersensitivity generally undergoes a slight or fair diminution.

Recovery in patients with erythema nodosum who have undergone such treatment has been constant and rapid. Fairly good results can be obtained in some cases of generalized acute miliary tuberculosis. The return to normal of the erythrocyte sedimentation rate in every case was much more rapid than in the controls.

* * *

In conclusion, from studies of the cases of tuberculous meningitis and the less evident cases of tuberculosis of the serous membranes, of hyperergy (erythema nodosum), and various other forms of tuberculosis, our clinical experience allows us to assert that the use of cortisone in the standard dose of 1 mg. per kg. per day orally and of 2 to 5 mg. of hydrocortisone intracisternally has remarkable action. Of course, this therapy must be combined with moderate doses of streptomycin and isoniazid.

The results obtained with "Prednisone®," a cortisone preparation which has been somewhat more widely used in the last months in small doses (0.5 to 1.0 mg. per kg. per day orally) without evidence of the usual corticosteroid side-effects, have been good.

In serious cases the use of corticotropin by intravenous drip in moderate doses (10 to 15 units) in addition to cortisone orally seems to improve the efficacy of such treatment and rapidly increases its action.

I trust that I have been able to prove not only the harmlessness of cortisone and its derivatives as well as that of corticotropin in the therapy of tuberculosis, but also to point out the great importance of these drugs administered, of course, in moderate doses and always with the protection of active antimicrobials.

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## GENERAL DISCUSSION

Dr. Dressler: We will proceed to a discussion of the three papers of the morning, by Drs. Debré, Choremis, and Cocchi. Perhaps it would serve to start the ball rolling somewhat more quickly if I were to single out certain people and ask them to start.

Dr. Lincoln, I heard your name figure prominently in several of these talks, and I wonder if you would like to start the discussion.

Dr. Lincoln: I would like only to start, because there are so many interesting points in these three papers that I am afraid I could go on for a long, long time.

However, I would like to single out Dr. Debré's paper, because I think that his is a subject which is very acute at the present time. I think the single most important clinical question today in tuberculosis in children is whether we should universally apply treatment to all children with primary tuberculosis.

I entirely agree with what Dr. Debré has said, and I am delighted to learn that they are doing a randomized controlled series because I still feel that, although we know that isoniazid is a wonderful drug, and, although we are reasonably sure that, if given in adequate dosages and for a long enough time, it will prevent meningitis and miliary tuberculosis, I think a great many other questions still need to be discussed.

One question is the adequate dosage. Unfortunately we have seen recently on our ward a case of tuberculous meningitis in a child who had been treated for seven months with isoniazid by another group in our city who unfortunately believe in very low dosages. This child received only 2 mg. per kg. per day for a period of seven months. So, I think we have to be sure that the dosage is adequate. We, also, have to be sure about adequate duration of treatment.

Furthermore, I think we have to use prolonged follow-up in order to be sure that we really can prevent late complications, especially in chronic pulmonary tuberculosis. For instance, recently we have seen many of the children who have been successfully treated with streptomycin and isoniazid for miliary or primary tuberculosis, but it is too early to know what will happen to those children during adolescence.

Recently we had an opportunity to see one of our children who had been successfully treated in 1944 with Promizole® alone for three years for acute generalized miliary tuberculosis. She remained perfectly well, but recently, within a short period after menarche, she has developed an apical reinfection type of tuberculosis.

So, I think we still have to plan, as Dr. Debré has done in his group, and as the Public

Health Service in this country is planning to do, to follow these children for a long period of time.

DR. HATTIE E. ALEXANDER (New York, New York): I would gather from Dr. Debré's discussion that, even under circumstances of exposure of the child, he would prefer to wait until tuberculin hypersensitivity appears before starting isoniazid. On the other hand, I believe Dr. Canetti has been recommending, in areas of the world where there is such a high incidence of tuberculosis, the simultaneous start of BCG and isoniazid.

Dr. Schmidt's results, presented yesterday, suggested that a more lasting influence on the course of tuberculosis in monkeys after discontinuation of isoniazid therapy occurred in monkeys which had already had some tuberculosis before treatment. I would like to raise the question of whether Dr. Schmidt has analyzed this question in monkeys to the point where he can answer this: Is isoniazid much more effective in its lasting influence when tuberculin allergy has been established before its institution?

DR. SCHMIDT: The only answer I can give to Dr. Alexander's question is already contained in the data presented yesterday. I think these data showed quite definitely that in the infected animals which had apparently recovered under treatment from active pulmonary disease there were significantly fewer recrudescences of the disease than in the animals which were exposed to the possibility of infection, treated with isoniazid, and then after a period of time left on their own.

DR. J. PARK BIEHL (Cincinnati, Ohio): I would like to add another dimension to Dr. Lincoln's discussion.

Do we know that anything other than isoniazid alone is necessary in the treatment of childhood tuberculosis? We have a lot of information dealing with the emergence of resistance to isoniazid in adults, but I am not aware of any systematic study or any suggestion of the prevalence of isoniazid resistance, short of tuberculosis that has gone into cavitation, treated with isoniazid alone.

DR. LINCOLN: It is very difficult to study resistance in children because the population of the bacilli is usually very small, and we have very few children who have developed resistance to isoniazid. In our clinic we have very little experience with resistance to isoniazid because, except for the Public Health study, we always use isoniazid in combined therapy.

DR. ROBERT DEBRÉ (Paris, France): The question asked by Dr. Alexander is very important, and in my opinion at the present time it is not very clear. It seems preferable to begin treatment after the appearance of the allergy, and not before. When we observe a negative skin test in a child at the beginning of infection (after a few weeks), the results are not as good as when the treatment is begun before the allergy. We are of the opinion that it is a good time to start it then. It seems to me that Dr. Schmidt's experience with the monkeys is very important. It is possible that it is better to help them with antimicrobials when they are beginning to react to the infection, and not to treat them before allergy has appeared.

Concerning the problem of bacterial resistance, I had the same experience as Dr. Lincoln; we have seen very few cases of resistance to isoniazid among our children. No case can be suspected to be resistant. In our opinion, in the beginning of tuberculosis, with new and fresh lesions, the patient very seldom develops resistance to isoniazid. Of course, we have given PAS together with isoniazid to children.

DR. CHOREMIS: We absolutely agree with Professor Debré as he described the importance of the problem of therapy of childhood tuberculosis.

I think that today our therapeutic armamentarium, with all the modern chemotherapeutic agents, constitutes a major factor in the defense of the younger age groups against primary tuberculosis and its tendency to dissemination.

For our conditions in Greece we consider the following therapeutic scheme to be satisfactory: (*1*) In febrile cases of primary tuberculosis, regardless of age and type, the combined streptomycin-isoniazid treatment is indicated. Streptomycin is given during the febrile phase, 20 to 40 mg. per kg., and for one week after the fever subsides. We administer isoniazid, 10 to 15 mg. per kg., for a period of six months: 15 mg. per kg. of body weight during the first three months, and 10 mg. per kg. (or less, in older children) thereafter. (*2*) In nonfebrile cases the therapeutic management depends on the patient's age.

We do not treat children more than three years old if their only manifestation is a positive tuberculin reaction, while younger children (less than three years) with such a reaction are treated with isoniazid, 5 to 10 mg. per kg. of body weight.

However, if X-ray findings are present, then we apply the regular therapeutic regimen of streptomycin and isoniazid, independent of age.

We do not use PAS as a prophylactic or therapeutic agent even in such severe cases of tuberculosis as tuberculous meningitis or miliary tuberculosis. We consider our results from the application of the above-mentioned therapeutic regimen absolutely satisfactory.

Thus, of the 570 cases of primary tuberculosis, none was complicated with tuberculous meningitis or other dissemination. On the contrary, of the 227 tuberculous meningitis patients admitted in our clinic during the last three years, only one had been treated, and this one incompletely, for pulmonary tuberculosis six months before the onset of tuberculous meningitis.

DR. STEAD: Obviously a great deal hinges upon the time when therapy is started in the converter. The principal difficulty in this clinically, and obviously from the Public Health standpoint, is finding the converter after he has developed his allergy but before he has developed a pleural effusion or hilar nodes, and so on.

I wonder if there is any experience with depot tuberculin or some method of this type to detect the allergy when it first develops.

DR. RIST: Some investigations have been made in Scandinavia, particularly in Sweden, with regard to depot tuberculin, but I think it is fair to state that no practical method has so far been elaborated. I know, however, that Dr. William Feldman at the Mayo Foundation has taken an interest in this question lately. I do not know, however, whether he has any results.

I would like to say a few words with regard to the paper by Dr. Choremis. His findings may have an important bearing on the question raised by Dr. Canetti whether a BCG vaccination and a prophylactic treatment could be carried out simultaneously.

However, the figures of Dr. Choremis were extremely small, and when you operate with such small groups you have to apply an extremely careful technique. I would therefore like to know who read the local reactions and the tuberculin reactions, and whether the person or persons concerned had any knowledge regarding the group to which the children belonged when they read the different reactions.

DR. CHOREMIS: In our clinic we have treated more than 700 cases of tuberculous meningitis in the last seven years, so I have absolute confidence in my doctors and in their evaluations of allergy.

I agree that the number of children is perhaps very small, but the results are consistent. If they are true, they give us a key to the explanation of the role of allergy. I believe that in the future, perhaps, repeated experiments like this can be done. If they are confirmed, perhaps we can discuss them.

Why does a child with tuberculous meningitis die? I believe it is because the lesions which we find at necropsy are due to allergy. In the first week, with streptomycin, the picture is completely modified, and the child seems to be healthier; but if you examine the cerebrospinal fluid you will find it is pathologic. You will see the discrepancy between the clinical picture and the findings. Streptomycin in the first two weeks cannot change the pathologic findings of tuberculous meningitis. This was found in more than 100 cases that have been autopsied in Athens. The clinical picture shows the importance of this allergy.

DR. CANETTI: I want to speak on two points. The first one is Dr. Choremis' results; the other is that I would like to go back to what Dr. Alexander said.

I think Dr. Choremis' results on allergy inhibition by isoniazid in children are, of course, extremely important. They are important in spite of the fact that the number of cases is small, and although I am afraid tuberculin sensitivity has been tested by a rather low dosage. Five TU is a little low. With 10 TU—

DR. CHOREMIS: I said ten.

DR. CANETTI: That's a little better. In any case, the differences are so striking between the treated cases and the controls that I am sure it is inhibited to a great extent.

What happens to allergy very likely happens to immunity. I think Dr. Choremis' experiments provide not absolute evidence but a very great probability that isoniazid to a large extent hampers the development of BCG immunity. I would not say entirely; and it probably does not hamper it entirely because a certain quantity of bacillary bodies is injected, and we know that dead bacillary bodies can produce a certain amount of immunity; so this low degree of immunity would be there, but probably no more than that.

I would like to ask Dr. Choremis one question. He tested his children at something like eleven weeks after vaccination. Did he test some of them very long after that to see if stopping isoniazid resulted in the appearance of tuberculin sensitivity—which would bring it somewhat nearer to Dr. Schmidt's observation?

Concerning Dr. Alexander's question, I don't think there is any contradiction in Professor Debré's statement on the usefulness of treating patients after allergy has appeared and the proposal I made two days ago on associating vaccination by isoniazid-resistant BCG and prophylactic chemotherapy. It is the same sort of thing.

I think it may well be worth starting treatment after establishment of allergy. Why? Well, I think the fact that allergy has appeared proves that the inhaled bacilli did multiply in the body, because we know they would not otherwise have produced allergy. We know they must have multiplied to a certain extent. We all know that multiplying bacilli (and it was shown yesterday by Dr. Mitchison) are far more susceptible to isoniazid than dormant bacilli. So, I think treating patients who have just acquired allergy means treating patients who have bacilli in the multiplying state. This, of course, may well give better results.

What I proposed is something entirely different. The accent in my proposal is not on chemotherapy but on vaccination. What I proposed was to give a means of vaccination which would not be inhibited by chemotherapy with isoniazid. That can only be achieved by a BCG strain which is isoniazid resistant.

The question whether with such a vaccination chemotherapy should be started immediately or somewhat later is, of course, very difficult to answer. What would happen in such cases? Let's suppose our BCG-vaccinated child in the first weeks developed immunity. If no superinfection occurs, of course there is no need of giving any chemotherapy. However, we don't know if there is an opportunity for superinfection in the child's environment. If it is a heavily infected environment, I think that giving chemotherapy would be very advantageous. But could we detect the moment when a superinfection actually occurred and then start treatment?

This would be perhaps a logical thing, but I think it would perhaps be even more logical to try to arrest, in the beginning, any appearance of such superinfection. That is why I think it would be better to start chemotherapy from the beginning, provided, of course, the vaccine is not susceptible to the chemotherapy.

This does not mean that, when we administer chemotherapy alone (which, in fact, would be a true chemoprophylaxis), it should be given before the appearance of allergy.

Dr. Lack: I would like to touch on two aspects of cortisone activity. I say two because I fully appreciate that cortisone has many more actions than the two I am going to discuss. The first is its suppression of hypersensitivity.

Many years ago experiments by Gardner and others showed a temporal relationship between the appearance of lymphocytes in exudates and the appearance of hypersensitivity. What Gardner did was to introduce suspensions of tubercle bacilli intraperitoneally in normal guinea pigs. The reversal of the monocyte-lymphocyte ratio invariably coincided with the development of skin sensitivity.

Recent work that I have been doing on the evolution of tuberculous lesions in guinea pig and rabbit omenta reinforces the theory that antibody carried by lymphocytes may be responsible for cellular necrosis at the site of antigen. I suggest that the lymphocytolysis induced by cortisone may depress the carriage of antibody to the site of tuberculin inoculation.

The second point I would like to mention is the proteolysis which may sometimes be observed after cortisone treatment. Professor Choremis mentioned this in his paper. I produced a chronic infection in rabbits by intradermal inoculation with human bacilli.

When such rabbits have been kept for one hundred and fifty days, the skin lesion is no longer ulcerated. It is a firm nodule surrounded by a fibrotic wall, and foci in the lungs are also chronic epithelioid lesions well walled off with fibrous tissue.

When these rabbits are given cortisone on the one hundred and fiftieth day, a most striking softening of these foci occurs and the lung lesions are rapidly invaded by inflammatory cells. The rabbits then deteriorate in condition and die.

This fibrolytic effect of cortisone is the one which I think may contribute to those changes in the joints which I described yesterday. I want to take the opportunity of saying here that I am not deprecating the use of cortisone under antimicrobial cover, but merely emphasizing the danger of its use when the possibility of tuberculosis being present has not been considered.

I can well imagine situations in which this softening of chronic lesions may enhance the effect of antimicrobial therapy by allowing the tubercle bacilli to start multiplying and by making them more accessible to the drugs.

Dr. Ström: In discussing Dr. Choremis's paper, first I want to say that somehow I agree with Dr. Canetti. I have the feeling that the doses of tuberculin were perhaps a little small. On the other hand, I want to ask Dr. Choremis if he thinks there is a certain risk of desensitization with repeated tuberculin tests. As I remember, they were repeated once a week.

We know that one can get a certain degree of desensitization in that way. I have in mind the work of Fourestier and Blacque-Belair, who purposely tried to get their so-called "anergies." They used repeated fresh BCG vaccine.

Dr. Choremis: To be more certain, you must have a BCG test made to confirm the allergy. We not only tested our children with BCG, but we used living BCG tests and dead BCG bacilli. All the results are in agreement. I agree with you that the number of cases is small, but under our conditions it is very difficult to keep children who are three months old under observation. We began our work with sixty children, and at the end of our experiment only forty-three remained.

I will continue my experiments with cortisone and perhaps in the future I can publish these results also.

# PRESENT METHOD AND RESULTS OF TREATMENT OF TUBERCULOUS MENINGITIS[1]

ROBERT DEBRÉ[2] AND H. E. BRISSAUD[2]

Enough time has now elapsed to allow some evaluation of the contribution of isoniazid to the treatment of tuberculosis. As for tuberculous meningitis, 220 cases were treated in our clinic in 1952, 1953, and 1954; the follow-up period of one to four years justifies drawing several conclusions. Isoniazid has proved to be easier to use than streptomycin, better tolerated, and more diffusible. These advantages have resulted in much simpler treatment and improved results (table 1). This is shown by the comparison of the mortality rates during two different periods. Our earliest statistics consisted of the first 93 children treated for tuberculous meningitis, from the beginning of 1947, with streptomycin at high dosage given in short series with interruptions. Of these 93 children, only 27 survived. They are still perfectly well now, with a follow-up period of eight years, but they constitute only a 30 per cent success, the 66 other children having died. These first 93 cases are not included in table 1.

The second analysis (table 1 (*b*)) was made in January, 1952. The material analyzed consists of the results in 309 children who were treated with smaller doses of streptomycin and PAS. The drugs were given continuously and for a long time, intrathecal therapy being discontinued only when the cerebrospinal fluid had returned completely or almost completely to normal, which always took several months. Of these 309 patients, 124 died and 185 (60 per cent) recovered. In this long period (1947–1952), although some modifications of therapy were tried, no obvious improvement of the results was obtained, while the improvement was dramatic as soon as isoniazid was available.

This is shown in our third set of statistics, including 220 children treated with isoniazid as well as streptomycin and PAS according to the previous regimen, in the years 1952, 1953, and 1954 (table 1 (*a*)). Let us point out that this figure includes all children who received isoniazid at any period of their treatment, even at a moribund stage. Of these 220 children, 176 survived and only 44 died. Thus, the recovery rate suddenly reached 80 per cent. These figures include:

In 1952, 17 deaths and 69 survivals
In 1953, 14 deaths and 71 survivals
In 1954, 13 deaths and 36 survivals

In spite of such a remarkable improvement, we think the contribution of isoniazid is rather understated by these statistics, and it is only fair to evaluate the results differentially: in children who have been treated with isoniazid from the beginning of the illness; and according to the degree of severity of the disease, especially according to age groups, as the young age group has been shown to have a very bad prognosis.

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TABLE 1

TREATMENT OF TUBERCULOUS MENINGITIS WITH ISONIAZID
MORTALITY RATE WITH AND WITHOUT ISONIAZID
Children Admitted Before January 1, 1955. Results at November 1, 1955

(*a*) Children treated *with isoniazid* at any period of meningitis

220 patients → Dead 44 (20%)
220 patients → Alive 176 (80%)

(*b*) Children treated on our service with uninterrupted antimicrobial treatment without isoniazid

309 patients → Dead 124 (40%)
309 patients → Alive 185 (60%)

TABLE 2

TREATMENT OF TUBERCULOUS MENINGITIS WITH ISONIAZID
RESULTS OF ISONIAZID TREATMENT DEPENDING ON THE TIME AT WHICH ISONIAZID HAS BEEN GIVEN
Patients Admitted Before January 1, 1953. Results at November 1, 1955

| Patients Treated at First without Isoniazid | | Patients Treated with Isoniazid from the Beginning of the Illness |
|---|---|---|
| Isoniazid Added for Chronic Meningitis | Isoniazid Added in the Course of Evolution | |
| 29 patients: Dead 9 | 30 patients: Dead 6 | 27 patients: Dead 2 |
| 29 patients: Alive 20 | 30 patients: Alive 24 | 27 patients: Alive 25 |

In table 2 the differences in the results are shown, according to whether isoniazid was used from the beginning of treatment or only later. These results were obtained in 1952 because, since 1953, although most patients have received isoniazid from the beginning, the majority of them had their treatment started in various centers with various techniques. This makes the interpretation of the results somewhat difficult. In 1952, of 59 children who received isoniazid at a more or less late stage of disease, 15 died; while there were only two deaths among 27 children treated with isoniazid from the beginning of their illness.

In table 3 may be seen the considerable difference of mortality rates in the different age groups among the 220 children treated with isoniazid in 1952, 1953, and 1954.

Age is not the only important factor in prognosis. The state of consciousness on admission remains the valuable element of prognosis that we have previously stressed. All of the children, aged more than two years, who died were uncon-

scious on admission to this clinic. This demonstrates an important point that all practicing physicians should always keep in mind: a good prognosis depends on an early diagnosis.

Our present method of treatment has been dictated by our first results with isoniazid and by what is now known of its pharmacology. The drug shows inhibitory activity on *Mycobacterium tuberculosis in vitro* at very low concentrations (less than 0.03 γ per ml.), about tenfold less than for streptomycin. Isoniazid is highly diffusible in blood, and the cerebrospinal fluid concentration of the drug is about half that present in blood and is much higher than the minimal inhibitory concentration for tubercle bacilli *in vitro*.

Nevertheless, we have not dared in all cases to use isoniazid and PAS alone. In the year 1954 we applied this combination only in 16 cases which were not too serious. The other 33 were of such severity that we employed streptomycin as well. As treatment with isoniazid-PAS has been applied only to selected cases, we do not give our statistics as conclusive evidence for the superiority of this treatment without streptomycin. But we think that it does show that the treatment with isoniazid-PAS, with no intrathecal injections, can be used with good results, at least in cases in which the process is not too far advanced.

TABLE 3

TREATMENT OF TUBERCULOUS MENINGITIS WITH ISONIAZID
RESULTS ACCORDING TO AGE
Patients Admitted Between January 1, 1952, and January 1, 1955.
Results at November 1, 1955

| Age | Number of Patients | Dead | |
|---|---|---|---|
| Less than 2 years | 45 | 16 | (64.5% recoveries) |
| 2–5 years | 76 | 21 | (72.0% recoveries) |
| More than 5 years | 99 | 7 | (93.0% recoveries) |
| Total | 220 | 44 | |

TABLE 4

TREATMENT OF TUBERCULOUS MENINGITIS WITH ISONIAZID
RESULTS OF TREATMENT WITH AND WITHOUT INTRATHECAL INJECTIONS
Children Admitted Between January 1, 1954, and January 1, 1955.
Results at November 1, 1955

| Treatment Started with Intrathecal Injections | Treatment Started without Intrathecal Injections |
|---|---|
| 33 patients → Dead 11; Alive 22 | 16 patients → Dead 2*; Alive 14 |

* One of thrombocytopenic purpura; the other of the evolution of his meningitis (in spite of added intrathecal treatment with streptomycin, isoniazid, and PAS; 3 relapses had previously been influenced by viomycin therapy).

It may be seen in table 4 that, of the 16 children treated at first without intrathecal therapy, only 2 died, one of thrombocytopenic purpura; the other, of the evolution of the meningitis in spite of subsequently added intrathecal treatment with streptomycin, isoniazid, and PAS. Three previous relapses had been influenced by viomycin therapy.

Thus, our present treatment is as follows: isoniazid is the main therapeutic agent. We give at least 20 mg. per kg. per day with a maximum of 600 mg. per day in the older children. In small infants we give as much as 30 or even 40 mg. per kg. per day. It is given in four divided doses orally. We use the intramuscular route only in the presence of vomiting.

We give PAS by mouth, along with isoniazid at a daily dose of 300 mg. per kg., in one dose or two divided doses.

This treatment is given without interruption for at least ten to twelve months, and sometimes longer.

We give streptomycin from the beginning only in the patients with an abnormal state of consciousness, treated at a late stage. But we have sometimes added streptomycin secondarily to the treatment with isoniazid-PAS if we were not satisfied with the rate of improvement. We have discontinued streptomycin as soon as the child got better and carried on with isoniazid-PAS alone.

We give streptomycin at a daily dosage of 0.5 to 1.0 gm. in three intramuscular injections per day, and intrathecally 15 to 30 mg. injections once daily.

In comatose children, feeding is a very important problem. It has to be done by gavage, often over periods of several months, in order to maintain a good state of nutrition. It is a difficult nursing problem, which has to be met successfully, as consciousness can return to normal even after several months of coma.

Thus, we do not use a routine treatment in the sense that it is the same in all cases. We believe that the intensity of the therapy has to be somewhat proportional to the severity of the disease in each case. This approach permits the complete recovery of all children more than two years of age in whom the meningitis has not been diagnosed too late.

# THE MANAGEMENT OF SPECIAL PROBLEMS IN THE CHEMOTHERAPY OF TUBERCULOSIS IN INFANTS AND CHILDREN[1]

JOSÉ E. SIFONTES[2]

This paper is based upon observations of 555 infants and children hospitalized for tuberculosis at the Alejandro Ruiz Soler Sanatorium in Puerto Rico between December, 1952, and July, 1955. The mortality from tuberculosis during this period was 1.9 per cent (table 1).

The largest number of patients died of miliary and meningeal tuberculosis and had usually been brought to therapy in late stages of the disease. Many of the patients who died (table 2) and others who survived after a stormy course suffered from severe malnutrition, bronchopneumonia, hypoproteinemia, pyelonephritis, anemia, intestinal parasites, and other illnesses.

These facts introduce one of our most important problems in chemotherapy, the management of patients desperately ill with tuberculosis.

An important initial problem is to give accurate dosages to patients who are markedly undernourished. Since the weight on admission of 50 per cent of our patients is below the third percentile for their age, drug dosage based on body weight would probably be too low in that group of patients. For this reason we prefer dosage calculation based on body surface (1).

In markedly undernourished children, this method of calculation may result in doses two or three times higher than if based on body weight. We administer daily doses of approximately 150 to 200 mg. of isoniazid per $M^2$, 1 gm. of streptomycin per $M^2$, and 12 gm. of PAS per $M^2$.

The management of a child seriously ill with tuberculosis during the first month of therapy usually determines whether the patient survives or not. It consists not only of chemotherapy of tuberculosis, but also of vigorous supportive therapy.

Secondary infection may produce death when the tuberculous process is under control. We use the following approach to this type of problem: the isolation of the patient; the use of penicillin or, preferably, wide-spectrum antimicrobials; and the administration of gamma globulin. In many of our patients we believe that this approach has been the life-saving factor.

Supportive therapy should also include prompt correction of hypoproteinemia and severe anemia with blood transfusions and plasma. Patients who are dyspneic should receive oxygen. Many patients are unable to ingest sufficient amounts of food, and they should receive adequate amounts of fluids, calories, and vitamins through a polyethylene gastric tube. This phase may last for weeks or months, and dependence upon intravenous feeding may allow the patient to become debilitated and unable to combat his disease.

Some patients may vomit at the onset of tube feeding, but this may be con-

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TABLE 1

ANALYSIS OF MORTALITY FROM TUBERCULOSIS AMONG 555 INFANTS AND CHILDREN ADMITTED TO THE ALEJANDRO RUIZ SOLER SANATORIUM
December, 1952 to June, 1955

| | Number |
|---|---|
| Total number of deaths | 11 |
| Autopsies performed | 8 |
| Miliary tuberculosis and meningitis | 6 |
| Progressive primary | 3 |
| Reinfection far advanced | 1 |
| Miliary | 1 |
| Died within 24 hours (also included above) | 4 |

TABLE 2

NONTUBERCULOUS ILLNESSES OBSERVED AMONG ELEVEN INFANTS AND CHILDREN WHO DIED FROM TUBERCULOSIS

| | Number |
|---|---|
| Severe malnutrition | 5 |
| Bronchopneumonia | 4 |
| Hypoproteinemia | 2 |
| Pyelonephritis | 2 |

Others: Congenital hydrocephalus, Mongolian idiot, otitis media, mastoiditis, intestinal parasites

trolled if the causes of vomiting are investigated and eliminated; others, especially those who have gastroenteritis, may need fluids, electrolytes, and proteins by the intravenous route.

In regard to indications for chemotherapy, we believe that in any patient with progressive tuberculosis, whether it be locally progressive primary, reinfection, miliary, meningeal, osseous, or renal tuberculosis, there is no question of the desirability of chemotherapy. We prefer isoniazid and PAS as initial therapy, except when the patients are seriously ill, when streptomycin, PAS, and isoniazid are all used together.

On the other hand, in patients with asymptomatic active primary tuberculosis, there are more valid arguments against and for chemotherapy. When streptomycin was used in the therapy of these patients, we noted that it did not prevent the development of tuberculous meningitis. On the other hand, observations when using isoniazid in these patients suggest that it is possible to prevent tuberculous meningitis and other complications of primary tuberculosis by treating patients with active primary tuberculosis with isoniazid (2).

A factor against treating all patients with active primary tuberculosis, or perhaps all recent tuberculin converters, is the possibility that the bacilli may become resistant to the drug and that the patient may develop a progressive form of tuberculosis at a later date and may not be able to benefit from the drug. The final answer may not be obtained until the results of controlled in-

vestigations (3), such as the United States Public Health meningitis prophylaxis study, are available.

Toxic manifestations from chemotherapeutic agents are not common in the young. The chief problems are encountered when streptomycin and PAS are used. Streptomycin may destroy vestibular function; however, most patients compensate for this rapidly and effectively. More serious, and fortunately more rare, is eighth-nerve deafness associated with administration of streptomycin by the intramuscular route.

In regard to streptomycin by the intrathecal route, we have studied a group of patients, including control subjects (table 3). We observed 73 patients with tuberculous meningitis, all of whom received isoniazid. Thirty-five patients received streptomycin by the intrathecal route and 38 were controls who received no intrathecal therapy. In the "intrathecal group" (table 4) 21 survived, and 12 of these were deaf. Among the 38 controls, 25 survived and 3 were deaf. One of these had bilateral chronic purulent otitis media. It may be concluded that patients with tuberculous meningitis fail to show additional benefit from streptomycin by the intrathecal route. Furthermore, if streptomycin is administered, deafness is more likely to occur.

The most disturbing toxic reactions from PAS have been gastrointestinal disturbances manifested by anorexia, vomiting, and diarrhea. We have not hesitated to discontinue PAS when such disturbances interfere with the nutrition of the patient. Often it is possible to restart PAS again by giving very small doses and gradually increasing the amounts until therapeutic doses are reached.

TABLE 3

TUBERCULOUS MENINGITIS

MORTALITY AND STAGE OF ILLNESS: RELATION TO THERAPY BY THE INTRATHECAL ROUTE

(Patients Admitted to Several Hospitals)

| | Intrathecal Streptomycin | | No Intrathecal Therapy | |
|---|---|---|---|---|
| | Number of Patients | Per Cent | Number of Patients | Per Cent |
| Total | 35 | | 38 | |
| Stage at onset of therapy: | | | | |
| Early | 2 | 5.7 | 4 | 10.5 |
| Moderately advanced | 22 | 62.9 | 15 | 39.4 |
| Late | 11 | 31.4 | 19 | 50.0 |
| Survived | 21 | 60.0 | 25 | 65.8 |

TABLE 4

TUBERCULOUS MENINGITIS

INCIDENCE OF DEAFNESS AMONG SURVIVORS

| | Intrathecal Streptomycin | | No Intrathecal Streptomycin | |
|---|---|---|---|---|
| | Number of Patients | Per Cent | Number of Patients | Per Cent |
| Total | 21 | 100 | 25 | 100 |
| Deaf | 12 | 57 | 3 | 12 |

Isoniazid has not produced serious toxic reactions in any patient we have treated. On the other hand, we have observed hepatic damage and psychosis with iproniazid in a twelve-year-old boy. Viomycin has been well tolerated in a small number of patients whom we have treated with it. Allergic reactions to all of these drugs have been mild and transient.

Another problem in the chemotherapy of tuberculosis in infants and children has been bacteriologic positivity accompanied by persistent or progressive tuberculous lesions after a year or more of chemotherapy.

We have observed this type, mainly in patients with the reinfection type of tuberculosis. The incidence has been 10 per cent of 47 patients with far advanced and moderately advanced pulmonary lesions. *In vitro* drug-susceptibility studies revealed microbial resistance to isoniazid and streptomycin. In most cases cross-resistance between isoniazid and iproniazid was observed. In general, microbial resistance appears to be less common in children than in adults. With one exception, we have not observed microbial resistance *in vitro* or clinically in extrapulmonary tuberculosis, as manifested by relapse or recrudescence. This includes patients with osseous tuberculosis and tuberculous meningitis. The exception was in a patient with tuberculous otitis media.

The management of patients whose infection becomes resistant to chemotherapeutic agents depends upon what drugs the patient has been receiving. Streptomycin or isoniazid should be added if they have not been used previously. Iproniazid in our experience has rarely been effective when isoniazid has failed. Viomycin appears to be slightly less effective than streptomycin, but is valuable in cases of microbial resistance to other drugs. We believe viomycin was life saving in one of our patients. Pyrazinamide and cycloserine might also be of value. Surgery appears to be the only hope for some of these patients with persistent pulmonary lesions and positive bacteriology when therapeutic agents have failed.

* * *

In addition to the above general comments, each of the forms of tuberculous disease presents certain special problems.

### PULMONARY TUBERCULOSIS

Endobronchial tuberculosis due to bronchial compression by tuberculous lymph nodes is a difficult problem. Regardless of what type of chemotherapy is used or what attempts are made to relieve the compression through the bronchoscope, no satisfactory immediate results have been obtained except when a polyp was removed through endoscopy. In some cases, whether treated or not, the bronchial obstruction is relieved spontaneously; in others it persists and leads to atelectasis, bronchiectasis, and a permanently damaged lung. In these cases secondary pyogenic infections occur intermittently and require systemic and aerosol therapy with antimicrobial agents. An analysis of the results of a year or more of chemotherapy, including isoniazid, suggests that endobronchial disease may be responsible for a large percentage of sequelae (table 5). Mild persistent pulmonary lesions were commonly observed in patients whose disease

TABLE 5

RESULTS OF THERAPY OF PULMONARY TUBERCULOSIS IN INFANTS AND CHILDREN
Observations After One Year of Therapy

| | Complete Clearing | Asymptomatic Residual Infiltration | Extensive Lung Destruction-Atelectasis Bronchiectasis | Persistent Isolated Cavities | Presence of Acid-Fast Bacilli After 1 Year of Therapy |
|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| Progressive primary 79 patients | 50 | 39.8 | 2.5 | 7.7 | 0 |
| Reinfection; 47 patients | 20 | 47 | 23 | 10 | 10 |

improved and became arrested or inactive after therapy for progressive primary tuberculosis or reinfection tuberculosis. The majority of these patients were asymptomatic and did not have gross impairment of their pulmonary function.

On the other hand, lesions such as extensive atelectasis and bronchiectasis or persistent cavities were observed in 10.2 per cent of the patients with progressive primary and in 33 per cent of those with reinfection tuberculosis. Lobectomy, pneumonectomy, or segmental resection was the method of choice in dealing with these cases. We have also observed many of these children who did not have surgery because it was refused by parents or because they were poor candidates for operation. These children are doing surprisingly well. They tolerate exercise almost as well as other children, have frequent attacks of bronchitis; but, otherwise, live almost normal lives.

Thus we cannot say at present that surgery is best for all of these patients. In the future, the mortality from surgery will have to be weighed against the course of those patients who were not operated upon.

### TUBERCULOUS MENINGITIS

The most important problems occur as a result of block at the base of the brain or cerebral thrombosis which may account for permanent neurologic sequelae such as paralysis, mental retardation, and hydrocephalus. Cerebrospinal block, which was common when streptomycin by the intrathecal route was used, has become rare since this form of therapy was abandoned. In our experience, enzyme therapy as well as PPD have failed to modify block in tuberculous meningitis. Any type of therapy for block is extremely difficult to evaluate because in most cases the block tends to disappear spontaneously. Our most recent and promising attempts to diminish the incidence of block and neurologic sequelae include the use of steroid hormones (Prednisone®) early in the therapy of tuberculous meningitis.

### OSSEOUS TUBERCULOSIS

Osseous tuberculosis responds satisfactorily to chemotherapy and immobilization. After weeks or months of chemotherapy, including isoniazid, however, tuberculous abscesses and fistulas may occur in the tissue around the bone lesion. Once drainage occurs, the fistulas invariably heal within a few weeks. In these cases, we have obtained more rapid healing of the lesions by incision

and drainage of the abscesses as soon as fluctuation was observed, followed by periodic irrigations with streptokinase and streptodornase.

### TUBERCULOSIS OF THE SUPERFICIAL LYMPH NODES

Tuberculosis of the superficial lymph nodes presents a problem similar to that of osseous tuberculosis with respect to abscess formation. The management is similar, including irrigations with streptokinase and streptodornase. Most patients recover without need of surgical procedures, such as block dissection. At present our management of these cases consists of one year, or more, of chemotherapy, including isoniazid. So far we have observed no relapses after completion of therapy despite the fact that we have not performed dissection of the nodes.

### RENAL AND INTESTINAL TUBERCULOSIS

Renal and intestinal tuberculosis has ceased to be a problem since isoniazid became available. We have the impression that in many of our patients renal and intestinal lesions were suppressed by isoniazid before they were detected.

### TUBERCULOSIS OF THE SKIN

Tuberculosis of the skin has responded satisfactorily in all cases, except for the permanent scar formation that occurs. Tuberculous verruca cutis responds well to chemotherapy, but does so very slowly often after five months or more. This is important to realize because the dermatologist often becomes discouraged with chemotherapy before it is completed.

### TUBERCULOUS OTITIS MEDIA

Tuberculous otitis media presents a problem not only because it occasionally persists after a year or more of chemotherapy including isoniazid, but also because the flora usually changes to a purulent otitis media due to one or more pyogenic bacteria. These microbes are commonly resistant to all available antimicrobial agents and produce a chronic otitis media and mastoiditis. The high incidence (10 per cent) of purulent otitis due to pyogenic bacteria among our children with tuberculosis suggests that such otitis originated as a tuberculous otitis which later became secondarily infected.

One more problem which is important and controversial is the question of the amount of emphasis which should be placed upon bed rest as an adjuvant to chemotherapy. We have been unable to keep our patients in bed except when they are toxic and acutely ill, and we find that children who do not feel sick are unhappy when forced to remain in bed. In our hospital, the personnel is insufficient to maintain our patients in bed. This has forced us to observe children with all forms of tuberculosis, from meningitis to far advanced cases, running and exercising. So far we have seen no disastrous consequences from this forced experiment.

Even patients with Pott's disease, who, we know, sneak out of Bradford frames to play outside, seem to do well despite the insult to their diseased vertebrae.

Perhaps we should re-evaluate our concepts of bed rest in children with tuberculosis and let them be a little more free and a little more happy during their illness.

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# CORRELATION OF IN VITRO SUSCEPTIBILITY OF TUBERCLE BACILLI IN PATIENTS WITH MILIARY AND MENINGEAL TUBERCULOSIS WITH THEIR RESPONSE TO THERAPY

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As in other bacterial populations, emergence of tubercle bacilli resistant to streptomycin and to isoniazid is the result of selection of spontaneously occurring resistant mutants. In any sufficiently large microbial population susceptible to a given antimicrobial there arises a minute but constant proportion of organisms which can grow in concentrations which eliminate the masses. Since these cells are mutants, they differ genetically from the masses and also breed true; their multiplication after the others are destroyed may provide a population made up mostly or completely of resistant cells. Thus, a population emerges in a drug-resistant state.

It is now well established that this process may proceed according to two different patterns: the streptomycin pattern or the penicillin pattern, depending upon the antimicrobial which selects the resistant cells. In the penicillin pattern, emergence of resistance occurs only by a step-wise process. A single mutational step uniformly gives rise to a cell with only a low degree of resistance to penicillin; a high degree can occur only as a result of accumulation of a number of mutational steps. In the streptomycin pattern, a high degree of resistance can occur either in a step-wise fashion or by a single mutation.

It is significant for our discussion that the two most effective antituberculous agents, streptomycin and isoniazid, exhibit the streptomycin pattern of resistance. In patients treated only with streptomycin, emergence of a high degree of microbial resistance to streptomycin has been shown to play an important role in therapeutic failure. The experience with isoniazid appears to reflect a difference between isoniazid and streptomycin resistance in this respect. It has been reported that many strains found resistant to isoniazid *in vitro* appear to play no role in therapeutic failure in patients who receive only isoniazid.

In pyogenic infections, emergence of resistance to streptomycin and resultant therapeutic failure can be prevented by the simultaneous use of a second effective agent, which presumably works through a different mechanism of action. The latter assumption is made when the spontaneously occurring mutants resistant to one are normally susceptible to the other. This principle has been applied for some time in the treatment of miliary and meningeal tuberculosis in many pediatric institutions. Whether, in this way, emergence of drug-resistant tubercle bacilli can be eliminated in all types of lesions as a cause of failure has not been answered.

In the Babies Hospital this problem has been under investigation for several years. Populations of tubercle bacilli grown from patients with miliary and

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meningeal infection treated by two different regimens were studied for their susceptibilities to streptomycin and isoniazid.

Under the first regimen, streptomycin and PAS were used simultaneously from the start with or without a sulfone (Promizole®). Streptomycin was administered intramuscularly and intrathecally until the cerebrospinal fluid became normal by all criteria. Para-aminosalicylic acid and the sulfone were administered for at least one year.

Under the second program, streptomycin, isoniazid, and PAS were used simultaneously. Streptomycin was used intrathecally daily for one week in a dose of 25 mg., and intramuscularly (100 mg. per kg. of body weight per day) until the cerebrospinal fluid became normal. The isoniazid was administered in a dosage of 10 mg. per kg. of body weight per day. The administration of PAS and isoniazid by the oral route was continued for at least one year. In alternate patients, intrathecal purified protein derivative (PPD) was used.

The study was designed to determine not only whether emergence of microbial resistance can be prevented in miliary and meningeal tuberculosis, but whether an *in vitro* test of the drug susceptibility of tubercle bacilli grown can accurately indicate impending failure. For this purpose the populations of tubercle bacilli grown from the pretreatment fluids of each patient, e.g., the cerebrospinal fluid and gastric washings, were compared from the standpoint of drug susceptibility with the tubercle bacilli grown from the post-treatment fluids. Two different *in vitro* tests have been examined for possible value in detecting significant resistance, i.e., a degree of drug resistance which can be correlated with the patient's clinical response to therapy.

In the *in vitro* test which was first used, the minimal effective concentration was found which prevented the growth of 1,000,000 to 5,000,000 tubercle bacilli in Dubos fluid and on Dubos agar medium. The presence or absence of growth was recorded after fourteen and twenty-eight days of incubation. It soon became clear that this method detected the presence of organisms with a degree of resistance which has been considered significant. However, they were found in cultures from pretreatment as well as post-treatment body fluids. In fluid Dubos medium, tubercle bacilli with a much higher degree of drug resistance were grown. Only a minute proportion of the population formed colonies in the higher concentrations. Moreover, these few resistant cells appeared to occur at random. Serial isolates from the same patient could not regularly contain this minute proportion of resistant cells. The resistant cells found under these conditions have been interpreted as probably representing the normal proportion of spontaneously occurring resistant mutants. Their occurrence did not seem to be related in any way to the presence or absence of drug therapy or to the clinical response of the patient.

On the premise that these few resistant organisms represent a normal proportion of spontaneously occurring mutants, an attempt was made to prevent confusion caused by their presence by using a smaller testing population. For the past five years, two population sizes have been examined routinely, the 1,000,000 to 5,000,000 cell population used originally and a 100,000 to 200,000 cell population. Inocula of both sizes have been tested for their capacity to form colonies on the surface of Dubos agar in which various concentrations of strepto-

TABLE 1

MINIMAL EFFECTIVE CONCENTRATIONS (γ PER ML.) REQUIRED TO PREVENT GROWTH OF ONE TO FIVE MILLION (1) AND ONE HUNDRED TO TWO HUNDRED THOUSAND (2) TUBERCLE BACILLI FROM SOURCES DESIGNATED IN PATIENTS TREATED ACCORDING TO U. S. PUBLIC HEALTH SERVICE COOPERATIVE PROGRAM

| Patient | Source | Date | Streptomycin* | | | | Isoniazid† | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Days of Incubation | | | | | | | |
| | | | 14 | | 28 | | 14 | | 28 | |
| | | | (1) | (2) | (1) | (2) | (1) | (2) | (1) | (2) |
| P. W. | csf | 3-10-48 | 1.0 | 1.0 | 2.5 | 2.5 | 0.5 | 0.50 | 5.0‡ | 0.50 |
| (p-m) | vf | 10-4-48 | 10.0 | 5.0 | 50.0 | 10.0 | 0.5 | 0.50 | 5.0‡ | 1.00‡ |
| W. S. | gc | 3-6-48 | 1.0 | 0.5 | 100.0‡ | 1.0 | 0.5 | 0.50 | 50.0‡ | 50.00‡ |
| M. G. | csf | 6-29-48 | 1.0 | 0.5 | 1.0 | 0.5 | 0.5 | 0.01 | 0.5 | 0.05 |
| K. C. | csf | 1-3-51 | 1.0 | 0.5 | 2.5 | 1.0 | 0.5 | 0.50 | 5.0‡ | 0.50 |
| C. M. | gc | 5-2-51 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0‡ | 0.05 | 5.0‡ | 0.05 |
| | gc | 5-3-51 | 1.0 | 0.5 | 1.0 | 0.5 | 0.5 | 0.05 | 0.5 | 0.50 |
| J. B. | gc | 11-30-50 | 0.5 | 0.5 | 5.0‡ | 1.0 | 1.0 | 0.50 | 5.0‡ | 0.50 |
| | gc | 12-23-51 | 100.0 | 100.0 | 100.0 | 100.0 | 0.5 | 0.50 | 0.5 | 0.50 |
| A. Ta. | csf | 10-17-51 | 1.0 | 1.0 | 0.5 | 0.5 | 0.5 | 0.50 | 0.5 | 0.50 |
| | gc | 12-22-51 | 100.0 | 100.0 | 100.0 | 100.0 | 0.5 | 0.50 | 1.0‡ | 0.50 |
| F. M. | csf | 5-26-49 | 1.0 | 0.5 | 5.0‡ | 2.5 | 0.5 | 0.01 | 5.0 | 1.00‡ |
| M. A. | csf | 8-11-49 | 1.0 | 0.5 | 10.0‡ | 1.0 | 1.0 | 0.50 | 5.0 | 0.50 |
| C. B. | csf | 1-9-51 | 5.0 | 2.5 | 10.0‡ | 10.0‡ | 0.5 | 0.50 | 5.0‡ | 1.00 |
| G. G. | csf | 1-16-51 | 2.5 | 1.0 | 50.0‡ | 1.0 | 0.5 | 0.05 | 50.0‡ | 0.50 |
| | csf | 1-21-51 | 2.5 | 0.5 | 2.5 | 1.0 | 0.5 | 0.50 | 1.0 | 1.00 |
| J. L. | csf | 11-11-51 | 1.0 | 0.5 | 2.5 | 2.5 | 0.5 | 0.50 | 0.5 | 1.00‡ |
| | csf | 11-13-51 | 2.5 | 1.0 | 100.0‡ | 1.0 | 1.0 | 0.50 | 1.0 | 1.00 |
| P. R. | csf | 11-17-49 | 0.5 | 0.5 | 1.0 | 1.0‡ | 0.5 | 0.50 | 0.5 | 0.50 |
| R. C. | gc | 4-21-49 | 2.5 | 1.0 | 2.5 | 1.0 | 0.5 | 0.05 | 0.5 | 0.05 |
| | csf | 4-20-49 | 1.0 | 0.5 | 2.5 | 2.5‡ | 0.5 | 0.50 | 0.5 | 0.50 |
| | csf | 4-24-49 | 2.5 | 1.0 | 50.0‡ | 1.0 | 1.0 | 0.50 | 5.0‡ | 1.00 |
| J. C. | gc | 9-24-52 | 1.0 | 1.0 | 1.0 | 1.0 | 0.5 | 0.05 | 50.0‡ | 1.00 |

(1) and (2) = populations tested; gc = gastric content; csf = cerebrospinal fluid; vf = ventricular fluid; p-m = post mortem.

* Streptomycin = Dubos agar + streptomycin concentrations tested = 0, 0.5, 1.0, 2.5, 5.0, 10.0–50.0, 100.0 γ per ml. of medium.

† Isoniazid = Dubos agar + isonicotinic hydrazide concentrations tested = 0, 0.01, 0.05, 0.5, 1.0, 5.0, 50.0 γ per ml. of medium.

‡ Beside concentration listed indicates preceding concentration showed only 10 or less colonies; absence of ‡ indicates that 25 per cent or more of seeded population grew on preceding concentration.

mycin or isoniazid have been incorporated. The presence or absence of growth was recorded at fourteen and at twenty-eight days.

The populations of tubercle bacilli in the fluids cultured, with few exceptions, were too small to permit testing for drug susceptibility by seeding the specimen directly on medium containing isoniazid or streptomycin. It was therefore necessary to use secondary cultures. An effort was made to transfer a sample representative of the population grown to fluid

Dubos medium which contained Tween® 80. Subculture was repeated at seven-day intervals until a homogeneous suspension was obtained. Inoculum size was measured by colony counts obtained in pour-plate preparations in Dubos agar. When 0.1 ml. of a 5.0 ml. Dubos broth culture is subcultured at five- to seven-day intervals, the final viable population found in 0.1 ml. is approximately 1,000,000 to 5,000,000 cells. The following concentrations of streptomycin and isoniazid were examined:

Streptomycin, $\gamma$ per ml.—0.5, 1.0, 2.5, 5.0, 10.0, 50.0, 100.0

Isoniazid, $\gamma$ per ml.—0.01, 0.05, 0.5, 1.0, 5.0, 50.0

The influence of population size and incubation time on the minimal effective concentration needed to prevent growth of the same suspension of tubercle bacilli is apparent from table 1.

The data in table 2 represent a summary of the clinical findings in these patients treated on the regimen of streptomycin-PAS with and without sulfone therapy. Two results are shown for each population tested. In the first deter-

TABLE 2

MINIMAL EFFECTIVE CONCENTRATIONS FOR TUBERCLE BACILLI FROM PATIENTS WITH MENINGITIS AND MILIARY TUBERCULOSIS
TREATMENT: STREPTOMYCIN, PAS, AND PROMIZOLE®

| Patient | Source | Date | Streptomycin | | Isoniazid | | Clinical Result |
|---|---|---|---|---|---|---|---|
| | | | Test 1 | Test 2 | Test 1 | Test 2 | |
| P. W. | csf | 3-10-48 | 2.5 | 1.0 | 5.0* | 0.50 | D |
| (p-m) | vf | 10-4-48 | 50.0 | 5.0 | 5.0* | 0.50 | |
| W. S. | gc | 3-6-48 | 100.0 | 0.5 | 50.0* | 0.50 | D |
| M. G. | csf | 6-29-48 | 1.0 | 0.5 | 0.5 | 0.01 | D |
| K. C. | csf | 1-3-51 | 2.5 | 0.5 | 5.0* | 0.50 | D |
| C. M. | gc | 5-2-51 | 5.0 | 5.0 | 5.0* | 0.05 | L |
| | gc | 5-3-51 | 1.0 | 0.5 | 0.5 | 0.05 | |
| J. B. | gc | 10-30-50 | 5.0* | 0.5 | 5.0* | 0.50 | Deaf, retard.? |
| | gc | 12-23-51 | 100.0 | 100.0 | 0.5 | 0.50 | |
| A. Ta. | csf | 10-17-51 | 0.5 | 1.0 | 0.5 | 0.50 | Deaf,hemiplegia |
| | gc | 12-22-51 | 100.0 | 100.0 | 1.0* | 0.50 | Convulsions |
| F. M. | csf | 5-26-49 | 5.0* | 0.5 | 5.0 | 0.01 | L |
| M. A. | csf | 8-11-49 | 10.0* | 0.5 | 5.0 | 0.50 | S |
| C. B. | csf | 1-9-51 | 10.0* | 2.5 | 5.0* | 0.50 | D |
| G. G. | csf | 1-16-51 | 50.0* | 1.0 | 50.0* | 0.05 | L |
| | csf | 1-21-51 | 2.5 | 0.5 | 1.0 | 0.50 | Deaf |
| | csf | 7-17-51 | 5.0 | | 1.0 | | |
| J. L. | csf | 11-11-51 | 2.5 | 0.5 | 0.5 | 0.50 | |
| Recrudescence | csf | 11-13-51 | 100.0* | 1.0 | 1.0 | 0.50 | L |
| | csf | 3-16-55 | | 1.0 | | 0.05 | |
| P. R. | csf | 11-17-49 | 1.0 | 0.5 | 0.5 | 0.50 | Hemiplegia |
| R. C. | csf | 4-20-49 | 2.5 | 0.5 | 0.5 | 0.50 | D |
| | csf | 4-24-49 | 50.0* | 1.0 | 5.0* | 0.50 | |
| J. C. miliary | gc | 9-24-52 | 1.0 | 1.0 | 50.0* | 0.50 | L-Potts |

Test 1 = inoculum of 1,000,000-5,000,000; incubated 28 days.

Test 2 = inoculum of 100,000-200,000; incubated 14 days.

L = Living; D = Dead; S = Survival with some residual damage.

* Streptomycin = Dubos agar + streptomycin concentrations tested = 0, 0.5, 1.0, 2.5, 5.0, 10.0-50.0, 100.0 $\gamma$ per ml. of medium.

mination (Test I), approximately 1,000,000 organisms were seeded on Dubos agar containing different concentrations of streptomycin or isoniazid, and the presence or absence of growth was recorded after twenty-eight days of incubation. In Test II approximately 100,000 viable organisms were seeded and incubation was continued for only fourteen days.

The strain of tubercle bacilli grown post mortem from the ventricular fluid of *Patient PW* showed growth of 25 per cent or more when introduced as a "large" population in isoniazid concentrations of 10 γ per ml. but grew only in 2.5 γ per ml. when introduced as a smaller population. It would seem that there had been a selection of resistant mutants. However, the correct interpretation is far from clear because pretreatment strains from *Patients WS, CM, MA, CB,* and *GG* showed a range which was not significantly different except in the proportion of cells which showed resistance. The significant difference in minimal effective concentration measured by the two different drug-susceptibility tests

TABLE 3

MINIMAL EFFECTIVE CONCENTRATIONS FOR TUBERCLE BACILLI FROM PATIENTS WITH MENINGITIS AND MILIARY TUBERCULOSIS

TREATMENT: STREPTOMYCIN, ISONIAZID, AND PAS

| Patient | Source | Date | Streptomycin | | Isoniazid | | Clinical Result |
|---|---|---|---|---|---|---|---|
| | | | Test 1 | Test 2 | Test 1 | Test 2 | |
| J. T. | csf | 4-24-52 | 50.0* | 1.0 | 50.0* | 0.50 | L |
| | csf | 4-29-52 | 5.0 | 1.0 | 50.0* | 0.01 | |
| L. P. | csf | 6-12-52 | 10.0* | 2.5 | 1.0 | 0.01 | L |
| | csf | 6-18-52 | 5.0 | 2.5 | 50.0* | 0.01 | |
| G. H. | csf | 9-18-52 | 2.5 | 0.5 | 50.0* | 0.05 | D |
| B. B. | csf | 6-1-52 | 5.0 | 0.5 | 0.5 | 0.01 | L |
| + Miliary | csf | 6-6-52 | 2.5 | 1.0 | 0.05 | 0.01 | |
| N. M. | bc† | 4-24-52 | 2.5 | 0.5 | 50.0* | 0.50 | L |
| + PPD | csf | 4-28-52 | 5.0 | 0.5 | 50.0* | 0.01 | Deaf |
| H. F. | csf | 5-14-52 | 50.0* | 1.0 | 1.0 | 0.05 | L |
| + PPD | csf | 7-2-52 | 5.0 | 0.5 | 0.5 | 0.01 | |
| P. J. W. | csf | 6-5-52 | 10.0* | 1.0 | 50.0* | 0.01 | L |
| + PPD | csf | 6-9-52 | 0.5 | 0.5 | 5.0* | 0.01 | |
| L. B. | csf | 6-19-52 | 1.0 | 0.5 | 5.0* | 0.01 | Hemi- |
| + PPD | csf | 6-22-52 | 1.0 | 0.5 | 50.0* | 0.01 | plegia |
| E. Ri | csf | 1-15-53 | 1.0 | 0.5 | 5.0* | 0.50 | D |
| + PPD | gc | 1-15-53 | 0.5 | 0.5 | 50.0* | 5.00 | |
| L. C. | csf | 3-31-53 | 2.5 | 0.5 | 5.0 | 0.50 | D |
| + PPD | Cisternal f. | 4-1-53 | 10.0* | 0.5 | 50.0* | 0.05 | |
| J. To. | csf | 5-16-53 | 50.0* | 0.5 | 5.0* | 0.50 | L |
| + PPD | gc | 5-18-53 | 10.0* | 0.5 | 5.0* | 0.50 | |
| T. W. | gc | 3-21-52 | 5.0 | 0.5 | 50.0* | 0.05 | L |
| Mil. only | node | 6-17-52 | 5.0 | 0.5 | 50.0 | 0.05 | |

Test 1 = inoculum of 1,000,000–5,000,000; incubated 28 days.

Test 2 = inoculum of 100,000–200,000; incubated 14 days.

* Streptomycin = Dubos agar + streptomycin concentrations tested = 0, 0.5, 1.0, 2.5, 5.0, 10.0–50.0, 100 γ per ml. of medium.

† Blood culture

is striking. When the drug susceptibility was measured by Test II, only 2 patients, *JB* and *A Ta*, had strains of tubercle bacilli which exhibited a degree of resistance which might be expected to lead to therapeutic failure of streptomycin. These two strains were grown from gastric contents. More than 25 per cent of each populaton grew on medium containing 50 γ of streptomycin per ml. after fourteen days' incubation. No growth occurred on 100 γ per ml.; interval concentrations of streptomycin were not tested. In each patient the pulmonary lesion was shown by roentgenography to have spread during a period of a few months, but the patients continued to do well. They were treated with viomycin but a culture of sputum obtained at the time this drug was started failed to yield tubercle bacilli and the sputum has continued to show no tubercle bacilli. In each of these 2 patients a coalescent pulmonary lesion accompanied the miliary lesions.

In table 3 are listed the results of tests performed on populations cultured from patients treated with streptomycin, isoniazid, and PAS.

The comparison of the results of these two tests for assessment of increase in resistance, which might lead to therapeutic failure, suggests that in this therapeutic group also a population of 100,000 to 200,000 tubercle bacilli incubated on Dubos agar for only fourteen days offered a better index of susceptibility of the organisms to streptomycin and isoniazid.

In table 4 are listed the data obtained by Test II on strains from patients treated by one of our present regimens, streptomycin, isoniazid, and PAS, with-

TABLE 4

MINIMAL EFFECTIVE CONCENTRATION OF STREPTOMYCIN AND ISONIAZID, γ/ML., FOR TUBERCLE BACILLI ISOLATED FROM PATIENTS WITH MENINGITIS
TREATMENT: STREPTOMYCIN, ISONIAZID, PAS, WITHOUT PPD

| Patient | Source | Date | Streptomycin | Isoniazid | Result |
|---|---|---|---|---|---|
| J. T. | csf | 4-24-52 | 1.0 | 0.50 | L |
| | csf | 4-29-52 | 1.0 | 0.01 | |
| L. P. | csf | 6-12-52 | 2.5 | 0.01 | L |
| | csf | 6-18-52 | 2.5 | 0.01 | |
| B. B.* | csf | 6-1-52 | 0.5 | 0.01 | L |
| | csf | 6-6-52 | 1.0 | 0.01 | |
| L. C. | csf | 3-31-53 | 0.5 | 0.50 | D |
| | csf | 4-1-53 | 0.5 | 0.05 | |
| M. G. | csf | 6-27-53 | >100.0 | 5.00 | D |
| (p-m) | Spleen | 6-29-53 | >100.0 | 5.00 | |
| C. P. | csf | 7-8-53 | 1.0 | 0.01 | D |
| (p-m) | Brain | 8-13-53 | 1.0 | 0.05 | |
| J. G. | csf | 11-2-53 | 1.0 | 0.01 | D |
| (p-m) | Brain | 11-19-53 | 2.5 | 0.50 | |
| L. H. | gc | 6-14-54 | 1.0 | 5.00 | S |
| | csf | 6-24-54 | 1.0 | 5.00 | |
| M. A. | csf | 10-23-54 | 0.5 | 5.00 | D |
| (p-m) | Brain | 10-29-54 | 0.5 | 5.00 | |
| J. L.† | csf | 3-16-55 | 1.0 | 0.05 | L |

* Miliary as well as meningitis.

† Recrudescence.

TABLE 5

MINIMAL EFFECTIVE CONCENTRATION OF STREPTOMYCIN AND ISONIAZID, γ/ML., FOR TUBERCLE BACILLI ISOLATED FROM PATIENTS WITH MENINGITIS

TREATMENT: STREPTOMYCIN, ISONIAZID, PAS, WITH PPD

| Patient | Source | Date | Streptomycin | Isoniazid | Result |
|---|---|---|---|---|---|
| G. H. | csf | 9-18-52 | 0.5 | 0.05 | D |
| N. M. | bc | 4-24-52 | 0.5 | 0.50 | S |
| | csf | 4-28-52 | 0.5 | 0.01 | |
| P. W. | csf | 6-5-52 | 1.0 | 0.01 | L |
| | csf | 6-9-52 | 0.5 | 0.01 | |
| H. F. | csf | 5-14-52 | 1.0 | 0.05 | L |
| | csf | 7-2-52 | 0.5 | 0.01 | |
| L. B. | csf | 6-19-52 | 0.5 | 0.01 | S |
| | csf | 6-22-52 | 0.5 | 0.01 | |
| E. R. | csf | 1-15-53 | 0.5 | 0.50 | D |
| | gc | 1-15-53 | 0.5 | 0.50 | |
| J. T. | csf | 5-16-53 | 0.5 | 0.50 | S |
| | gc | 5-19-53 | 1.0 | 0.01 | |
| J. O.* | gc | 6-7-53 | 1.0 | 0.01 | |
| | csf | 6-7-53 | 0.5 | 0.01 | L |
| | csf | 1-19-54 | 0.5 | 0.05 | |
| C. R. | csf | 6-29-53 | 0.5 | 0.05 | L |
| G. R. | csf | 8-7-53 | 1.0 | 0.01 | S |
| | gc | 8-9-53 | 1.0 | 0.01 | |
| R. M. | csf | 11-25-54 | 0.5 | 0.05 | L |
| | gc | 11-28-54 | 1.0 | 0.05 | |

* Recrudescence.

out PPD. In one patient's strain a high degree of resistance to streptomycin was demonstrated before and after treatment. This patient died within forty-eight hours. While there was no known contact with a tuberculous person under therapy, this question merits a more thorough study. These strains also showed what would seem to be a significant degree of resistance to isoniazid. What has been considered a significant degree of resistance to isoniazid was found for the first and last strains of 2 other patients, one of whom died; the interpretation of these degrees of resistance to isoniazid requires more information. These strains are now being examined for catalase activity which may throw some light on this question.

In table 5 are listed the results of drug-susceptibility as measured by Test II of the first and last strains of tubercle bacilli cultivated from patients treated by streptomycin, isoniazid, and PAS and, in addition, PPD intrathecally. The absence of any suggestion of a significant degree of resistance in strains from these patients is interesting. The lower mortality here than in the group receiving the same treatment except for the absence of PPD is also interesting. Its meaning must await a larger experience and a longer follow-up period. However, the fact that PPD was withheld until one week after the start of treatment weights the results in favor of the PPD-treated group.

Emergence of resistance to isoniazid and to streptomycin during treatment has been demonstrated in populations of tubercle bacilli from the cerebrospinal fluid of only one patient whose cultures have been studied by either of the two drug-susceptibility tests described. The random appearance of a few cells showing a significant degree of resistance has been encountered in cultures of cerebrospinal fluid obtained before treatment as frequently as in post-treatment cultures. The misinterpretation which may follow demonstration of a normal proportion of resistant cells can be largely avoided by using a population of 100,000 seeded on a solid medium. The probability that any of the normal proportion of spontaneously occurring high-degree resistant mutants will be present is very low. Our experience suggests that use of a fourteen-day incubation period further decreases this probability and at the same time will not fail to demonstrate a significant increase in resistance during treatment. However, strains of tubercle bacilli from a larger series of patients studied over a longer period will be needed to establish this point.

The need to test tubercle bacilli grown from fluids prior to treatment will probably become greater as the number of persons under treatment increases. The culture grown from the cerebrospinal fluid of one patient before treatment with streptomycin, isoniazid, and PAS exhibited a high degree of resistance to streptomycin. The strain was not inhibited by streptomycin concentrations of 100 $\gamma$ per ml., even when the smaller inoculum and fourteen-day incubation period were used. This would seem to represent an example of an infection transmitted by an individual in whom emergence of microbial resistance to streptomycin had occurred during treatment. Whether growth in 1.0 $\gamma$ of isoniazid but not in 5 $\gamma$ per ml. represents a degree of resistance beyond that shown by the normal proportion of spontaneously occurring resistant mutants will require more knowledge for an answer. The cerebrospinal fluid cultures from 3 patients prior to treatment showed this minimal effective concentration.

Emergence of resistance (50 $\gamma$ per ml.) was demonstrated in the populations of tubercle bacilli grown from a pulmonary focus in 2 patients treated with streptomycin-PAS. The character of the pulmonary lesion appears to play an important role in determining whether or not there will be emergence of resistance.

Even though the appearance of resistant cells, which probably represent a normal proportion of spontaneously occurring mutants, cannot be shown to be correlated with the clinical response of these patients, who received three effective agents, they can be expected to play a different role when streptomycin or isoniazid is used as the only treatment. This experience with streptomycin is well known. The potentialities for emergence of a degree of resistance to isoniazid which reduces therapeutic efficacy in miliary and meningeal tuberculosis must await a longer period of study.

Emergence of resistance to streptomycin and isoniazid would seem to be a rare cause for therapeutic failure in tuberculous meningitis or typical miliary tuberculosis when they are used simultaneously from the beginning of treatment. Even when tubercle bacilli are grown from the cerebrospinal fluid for

many consecutive weeks or at widely spaced intervals when recrudescences occur, emergence of resistance has only rarely been demonstrated. When drug-susceptible tubercle bacilli persist they may be protected *in vivo* from the action of streptomycin and isoniazid which otherwise might select the spontaneously occurring resistant mutants. On the other hand, they may represent organisms in a physiologically insusceptible state comparable with those organisms which are responsible for latent pulmonary infection.

## GENERAL DISCUSSION

DR. DRESSLER: Thank you, Dr. Alexander. As in the last discussion period, we will call upon some member of our guest audience to start the discussion. I would first ask Dr. Platou to comment on Dr. Sifontes' paper.

DR. PLATOU: Having seen some of the work Dr. Sifontes is doing in San Juan, I can do no more than commend its quality. I particularly admire his attitude toward early, free ambulation in most forms of tuberculosis in children.

DR. EDITH LINCOLN: I think Dr. Sifontes' paper shows the necessity for good pediatric care of children with tuberculosis. So, as Dr. Johnston said yesterday, we treat the child with tuberculosis, not tuberculosis in the child.

DR. DEBRÉ: I wish to say that it is difficult to discuss the problem of tuberculous meningitis *in toto*. There are very important differences between different groups of cases.

In the ordinary cases, when the child is treated early in the disease, one can give treatment solely by the oral route and obtain very good results. But if the child comes in later in bad condition, it is perhaps better to continue with the intrathecal treatment as we have done in the past.

On the other hand, I must say that until four years ago we had never had deafness: we never use dihydrostreptomycin, and we give a small dosage of streptomycin to our children.

DR. DRESSLER: What do you think about this business of letting children run about actively with Potts' disease, Dr. Lack?

DR. LACK: I have no views about that from any direct experience. I think I said enough yesterday about the ill effects of immobilization on the skeleton and the permanent damage that can be done to children when they are immobilized for long periods. The effect of a prolonged stay in the hospital on the parent-child relationship is one that has bothered us very much in the orthopedic hospital where we have had children in for as long as seven years.

We have come to the conclusion that the child who is what a nurse calls "a good patient" is often a difficult child when he gets back home, and the child who rebels against the regimen of the hospital clings to his home background, and usually gets on better when he returns there.

DR. ALEXANDER: I would like to suggest that you call on Dr. Middlebrook. I thought we had a pretty good test for assessing susceptibility to antimicrobials until I visited Dr. Middlebrook's laboratory just before the symposium. This whole problem is even more complicated than I described in my paper. I would like to have him tell you what I am talking about.

DR. MIDDLEBROOK: Well, thank you very much, Dr. Alexander. Of course, instead of talking about it here—I have great difficulty in talking abstractly about something with which I am intimately familiar, it is operationally too difficult—every one who might wish is invited to come to the laboratory when the time is convenient for you, particularly tomorrow afternoon. We can present brief demonstrations of the techniques that happen to be used in this hospital for the study of bacterial populations and of mycobacteria in general.

I would like to take this opportunity to comment on an aspect of the treatment of tuber-

culosis which, in my opinion, has been too little emphasized. In particular, I would like to stress a phenomenon concerning which there has, to my knowledge, been no discussion whatsoever in the tons—well, pounds—of literature that have been published on the chemotherapy of tuberculosis.

This is just one aspect of the problem, but it seems to be one that has too long escaped our attention.

I know Dr. Alexander's experience with other infectious disease would make it possible for her to comment further with regard to the broader application of this phenomenon and its clinical implications.

There is such a thing in the chemotherapy of tuberculosis as the persistent excretion of tubercle bacilli which are drug susceptible by *in vitro* test. As Dr. Mitchison and Dr. McDermott so clearly pointed out yesterday, dormant tubercle bacilli, bacilli that are not multiplying, are certainly fantastically less susceptible to the sterilizing activity of the antimicrobial agents which have sterilizing effects on mycobacteria.

(Parenthetically, I hate to use the term, the tubercle bacillus. I am convinced this is operationally misleading, the singular use of the test is operationally misleading practice, with all apologies to Dr. Canetti whose title for his recent book was *The Tubercle Bacillus*. They vary enormously. They vary as widely as strains of pneumococci or of proteus vulgaris.) Drug-susceptible organisms excreted for long periods of time during the chemotherapy of an infectious disease can mean either of two things, it seems to me. First, one valid explanation views them as drippings from lesions in which the bacilli have not been multiplying during the period of chemotherapy but which suddenly open and permit their extrusion to a point where one can collect and identify them as drug-susceptible organisms. Or, second, there may be a failure to deliver adequate concentrations of the antimicrobial agent to the multiplying parasites.

If one will look at the tables of the Veterans Administration studies on chemotherapy of tuberculosis through the years, and if one picks out isoniazid alone, when it was used in a dosage of 300 mg. a day for adults, it will be surprising to see something which has had to be dug out of the material. That is, that at the six- to eight-month period after the initiation of chemotherapy with isoniazid alone or even with isoniazid and streptomycin, between 15 and 30 per cent of the patients who are still excreting quite large numbers of tubercle bacilli are excreting isoniazid-susceptible tubercle bacilli.

It does not mean that one patient does it at one time and another one at another time. The same patient persists in excreting drug-susceptible organisms.

Now, if we view these agents as bacteriostatic agents, as many clinicians have tended to do, then this is not a problem difficult to understand. We immediately believe that we have an explanation for it. But, I might say that for the past three years we have been trying to demonstrate a bacteriostatic activity of isoniazid against tubercle bacilli *in vitro*. We have been completely unable to do so. Isoniazid is either a sterilizing agent or it has no effect on tubercle bacilli. Dr. Mitchison emphasized this yesterday. I feel it is very worth while emphasizing it at the clinical level, the practical level of chemotherapy, which he didn't have time to do.

Isoniazid either sterilizes or it does nothing, and furthermore, isoniazid sterilizes at a rate which is more or less constant regardless of the concentration of the drug in the environment of the multiplying bacterial cells, provided the concentration is above a certain minimal concentration.

This means a great deal. These simple facts mean a great deal so far as the chemotherapy of tuberculosis in practice is concerned.

Now, one says immediately, well, that is *in vitro*. That is in the test tube. Surely it is in test tubes. But it should mean this, at least, to the clinician. It means that he should reevaluate his concepts of chemotherapy at least to the point of accepting the possibility of other protocols of treatment which take into consideration this new concept—thinking of these drugs as bactericidal agents for *multiplying* tubercle bacilli—and empirically determining whether or not it has any value when applied at the clinical level.

Dr. Alexander's presentation, I think, is enormously important. She isolates drug-

susceptible bacilli at intervals after treatment is begun. It is important to know how often they are isolated. They may only be "drippings." But she also mentioned patients with clinical relapses at the time the organisms were isolated.

I would like to know how often these clinical relapses occur and whether or not you attach any significance to the number of tubercle bacilli excreted—because these drippings are usually small in number.

We have actually seen patients—I think Dr. Dressler can tell you more about them—who apparently have ruptured a small necrotic focus and suddenly have a single positive sputum with drug-susceptible bacilli.

I know Major Morse has observed this phenomenon in a patient on Colonel Weir's service at Fitzsimmons. After a long series of negative smears and cultures on a patient under active chemotherapy, a smear was observed to be strongly positive, and the culture was also positive with drug-susceptible organisms. Within a few days, another sample of sputum was taken and no organisms were found from then on in the patient's sputum specimens.

This would be a large "dripping." This would be the result of excretion of a mass of drug-susceptible organisms from a necrotic focus, dormant during the previous period of chemotherapy. On the other hand, the persistent excretion from open cavities of drug-susceptible tubercle bacilli is a phenomenon which certainly hasn't been given any attention whatsoever in the chemotherapy of tuberculosis.

Dr. Choremis: In relation to the paper by Dr. Sifontes, I have the following to add. The great importance of pyogenic meningitis was not emphasized enough. During the early period of treatment when the children were not duly isolated, this severe complication was observed and has even provoked the death of some patients.

Infections occurring concomitantly with tuberculous meningitis, such as measles or mumps, do not necessarily make the tuberculous meningitis more serious; but the tuberculous meningitis may have a serious effect on chickenpox when these two diseases occur concomitantly.

Finally, I want to report two cases of cholesteatoma that occurred in children who had been cured of tuberculous meningitis for three years.

Both children were operated upon, and biopsy of the lesion was performed—of the cholesteatoma in the spine—and it was confirmed by biopsy and histologic findings. Because of the rarity of this occurrence, we believe this is probably attributable to chronic irritation of the meninges.

Dr. Lack: I have a little experimental evidence on changes that occur among tubercle bacilli exposed to concentrations of streptomycin and of isoniazid which are just sublethal. What we did was to take those cultures in titrations of streptomycin and isoniazid inhibition levels, which were just sublethal (just below the level of suppressing all bacterial activity) and culture these organisms on to Dubos agar plates containing that same concentration of the antibiotic. We then observed these cultures under phase contrast microscopy in the hot box. We found that multiplication of these bacilli occurred at these concentrations of the drug, but that the method of growth differed from the control cultures of the same strains in Dubos agar without the drug.

The strains grow in characteristic corded pattern, but the strains grown with these sublethal concentrations of drug do divide. That can be seen very clearly by studying them with phase contrast in the hot box, but they divide into shorter forms. That is, the individual bacilli do not grow as long as normal bacilli, but division does occur and growth tends to be in rather disoriented fashion. The difference in size may come down from a length of 7 mμ to 2 or 3 mμ. But even at that size, when these organisms are put into guinea pigs, multiplication can take place, although it takes much longer for the disease in the guinea pig to develop.

Dr. Mitchison: I would like to make one or two comments on Dr. Alexander's paper and on the comments Dr. Middlebrook made later.

First of all, although we know quite a lot about the way in which drug-resistance emerges in the case of streptomycin, in fact, we know very little about the similar process for isoniazid.

It is almost entirely dependent upon analogies between streptomycin and isoniazid.

It would be of very great value if somebody would carry out some of the critical genetic tests such as, in particular, replica plating or, at least, fluctuation tests with isoniazid.

Furthermore, there is no evidence at all that resistance to PAS, for instance, emerges by the same mechanism. However, if we do assume that resistance to streptomycin and to isoniazid emerges by similar patterns, that is to say, that existing mutants merely grow out, we would expect, in susceptible strains that have never come into contact with the drug, a very wide variation in the numbers of mutants to be anticipated in different colonies—much more than would be expected under ordinary circumstances.

For this reason we have to set a limit in our test at a rather higher level than we would if we were just counting red cells or something of that sort.

Now, I would suggest that Dr. Alexander's test still does not, even with her smaller inoculum, significantly distinguish between mutants and the bulk of the susceptible population.

I would suggest that the degree of variation, even with her smaller inoculum, is much too great, and that she should either decrease the size of the inoculum or increase the minimum number of colonies that she considers significant.

In our tests we accept 20 or fewer colonies as being insignificant. Some people take only 10 or 20 thousand organisms as their inoculum size and accept any colonies growing on medium containing drugs as drug resistant. Either of these is perfectly satisfactory.

I would comment on the question that Dr. Middlebrook raised, which, I think, of course, is one of the most fundamental and important questions at the moment in chemotherapy. I believe it is exceedingly difficult to get any clear-cut evidence whether antimicrobials really saturate the lesions or whether there are foci in which they are simply not active. I would suggest, however, that in most studies on pulmonary disease the evidence is slightly in favor of the saturation. The sort of evidence is that, if, for instance, streptomycin failed to penetrate into quite a high proportion of lesions, you might very well expect that isoniazid would penetrate better. In this case there would be areas within the lesions where only isoniazid was active, and you would, therefore, expect to get a very high incidence of strains resistant to isoniazid and susceptible to streptomycin.

Now, in actual fact, this does not occur with streptomycin and isoniazid in the usual dosage (streptomycin, 1 gm. per day). In the same way, if there were multiple-step production of streptomycin resistance, you would expect to get mutants which did not correspond in their degrees of resistance to those you can produce *in vitro* by single-step mutations.

In actual fact, we have shown that the mutants that you can get from patients do correspond very well to what you can get by single-step selection *in vitro*.

That is a second point of evidence that at least the vast majority of the multiplying population is exposed to streptomycin. However, I think in meningitis, in particular, the position may be very different.

There may very well be areas of chronic inflammatory tissue in which tubercle bacilli are essentially intracellular parasites in which slow intracellular growth can occur beyond the reach of the streptomycin.

According to this reasoning, one perhaps could expect that intrathecal streptomycin would be more effective than intramuscular streptomycin, and there is some evidence that this is so; and also that isoniazid would be a very much more potent agent in tuberculous meningitis than streptomycin, of which there is also considerable evidence. Thank you.

DR. ALEXANDER: First, I would like to comment on Dr. Middlebrook's discussion. He raised the question whether a comparable phenomenon has been encountered in inflammatory lesions with pyogenic organisms, that is, persistence of drug-susceptible organisms, and I would say I know of no such analogy.

DR. MIDDLEBROOK: Sulfonamides. Penicillin also.

DR. ALEXANDER: I would like to have you comment later on what you had in mind. It is true that there has been clinical recrudescence when we have continued to isolate tubercle bacilli even though they are not resistant. However, I would like to emphasize the fact that virtually all of the patients in whom there has been persistence of the susceptible tuber-

cle bacilli belong to the old era prior to isoniazid and that, at least in our hospital, during isoniazid therapy, we have had only one patient who has had persistent susceptible organisms during therapy; that was for a period of seven weeks.

It is very interesting that this particular patient finally made, as far as we can tell, a complete recovery. But the question does come up. There is one more possibility, I think, of an abscess or tuberculoma which softens and discharges tubercle bacilli. The organisms have not been in contact with streptomycin, and may come through the cerebrospinal fluid very rapidly and be planted out of contact with the drug before the bactericidal action can take place.

I would, therefore, like to hear Dr. Middlebrook discuss the time needed for the bactericidal action of streptomycin as well as of isoniazid; and again I would like to emphasize the point that under modern therapy—and I am sure that you are all using the one we are using at present, with some modifications—sterilization or at least our inability to culture tubercle bacilli occurs very rapidly during treatment.

Our cultures are uniformly sterile within less than seven days after starting chemotherapy, and our method for culturing tubercle bacilli is a very sensitive one.

Dr. Lack's discussion is very interesting, and I wonder if it is possible that these changes, these organisms which are changed morphologically, might possibly represent the second-step resistant mutants, and that these cells have two changes genetically—the morphology as well as the resistance. This leads me to ask whether you subsequently tested the sensitivity of these altered morphologic forms.

In relation to Dr. Mitchison's remark, I believe that we must also examine smaller populations. We are planning to do that simultaneously with larger populations in order to get an answer to that question.

In relation to the pattern of selection of these mutants by isoniazid, Dr. Szybalski at Cold Spring Harbor has published studies on emergence of resistance. As far as we know, it isn't the organism which determines the pattern, but the particular drug which selects the organism. Thus, it has been demonstrated that for any one strain of organism an antimicrobial brings out a certain specific pattern.

Dr. Mitchison: Dr. Szybalski worked with *Mycobacterium ranae*; it is different from *M. tuberculosis*. There might very well be a difference in mode of action of isoniazid on the two types of mycobacteria and possibly a different mode of acquisition of drug resistance.

Dr. Middlebrook: There *is* a different mode of drug resistance—fantastically different.

Dr. Koch-Weser: I just wanted to say one thing about Dr. Mitchison's comments. It certainly is true and the evidence points out that isoniazid penetrates tuberculous lesions better than streptomycin; but, as he himself pointed out yesterday, in the absence of oxygen, the antimicrobial activity of these drugs is very markedly decreased, if present at all. So it seems to me, if antimicrobial activity is not present, certainly resistance to the drug cannot appear.

It seems to me quite possible that the so-called "drippings" or the presence of drug-susceptible organisms might come from those badly oxygenated lesions, and can be present in the patient after long periods of chemotherapy.

I might say to Dr. Lack's remarks that those short, fat tubercle bacilli which are acid fast and which are drug susceptible can actually be produced in flasks in rarefied oxygen surroundings. If one puts nitrogen in culture flasks and decreases the oxygen concentration, such morphologic forms of tubercle bacilli can be produced. If inoculated into guinea pigs, one can isolate normal bacilli from those animals at a later date.

Dr. Kendig: I should like to direct two questions to Dr. Sifontes. The first one: What is the evidence that one must continue antimicrobial therapy for at least one year? In our cases of tuberculous meningitis, since the advent of isoniazid, not a single one of our survivors has had antimicrobial therapy for as long as a year. There has been no instance of relapse.

The other question I should like to ask deals with the question of block and tuberculous meningitis, and, while I understand he is using steroids early in the course of treatment at

the present time, if one does use the steroids, when does one decide whether or not the cerebrospinal block will clear spontaneously?

Dr. Sifontes: In regard to the first question about the duration of therapy, I think we have evidence first from patients with open pulmonary lesions. Very few of the children with pulmonary lesions become inactive with less than one year of therapy.

You always see gradual regressive changes, and I think it is well accepted that you should continue therapy for six months after you reach that point in chemotherapy when there are no changes in pulmonary lesions and no cavity.

In addition, we have seen no relapses in tuberculous meningitis in patients who have received one year or more of chemotherapy. On the other hand, we have seen relapses in patients who have received less than a year of therapy. The patient we have seen who had the longest duration of therapy and relapsed did so after ten months of therapy, including isoniazid. It was not in our hospital.

In regard to the other question, it is a very difficult one. The question is, When does one decide that the block is not going to clear spontaneously? Is that it?

Dr. Kendig: The point was made that the block usually clears spontaneously. If you don't use a steroid earlier, when do you decide to use a steroid?

Dr. Sifontes: Right now, what we are doing is using the steroids systematically in all patients, routinely. We have not put ourselves in a position to test. We do have a group of patients to compare with a previous group, but I imagine that the main evidence of the presence or absence of block is elevation of the cerebrospinal fluid protein. Usually if you have an increasing protein to more than 300 mg. per 100 ml. you may consider that you have a block. Then you must decide that you are going to treat the block.

Dr. Herbert J. Kaufman (Basle, Switzerland): I have a question that is directed, I guess, to Dr. Debré and Dr. Lincoln. I wonder whether the things that were mentioned are touching upon the same problem. Dr. Debré, as his paper was entitled, talked about therapy of primary tuberculosis. Dr. Lincoln, as I understand, was emphasizing the Public Health study, and this is entitled "Meningitis Prophylaxis Study." On the other hand, Dr. Debré is treating with PAS and isoniazid, and in a higher dosage than we here are using for taking care of our primary complexes or converters who are supposedly being treated to prevent meningitis. I would just like to ask you the question whether we treat —or, let's put it differently—whether we have a curative effect if we treat with isoniazid as it is being done at this present time, or whether this is really only preventive therapy. Of course, we don't know the answer yet. We will take care of the problems that may subsequently arise.

Dr. Debré: It seems to me to be impossible to answer the question. The answer is for the future. At the present time we do it with the best intentions, but we will have to wait and see.

In my opinion, we can hope to have a very curative therapy, but it is only a hope.

Dr. Lincoln: Actually, what we are doing, I think, in the Public Health Service study is mainly to treat children with asymptomatic tuberculosis. That was the chief object of the study, to see whether we could uniformly prevent meningitis in the treated group and, only incidentally, to study the course of treated local primary tuberculosis and the incidence of other complications.

## Session 6

Friday Afternoon, November 11, 1955

HASKELL COHEN, *Honorary Chairman*

ARTHUR ROBINSON, *Moderator*

# THE COURSE AND PROGNOSIS OF ENDOBRONCHIAL TUBERCULOSIS IN CHILDREN[1, 2]

EDITH M. LINCOLN,[3] LEONARD C. HARRIS,[3] SOMCHAI BOVORNKITTI,[3] AND ROSARIO CARRETERO[3]

The prognosis of primary pulmonary tuberculosis has been altered materially by antimicrobial therapy. In the Chest Clinic of the Children's Medical Service of Bellevue Hospital in New York City the death rate of primary tuberculosis was only 1 per cent in the years 1952 through 1954 as contrasted with a mortality rate of 21.5 per cent in the years 1930–1946 before the advent of streptomycin. Moreover, specific therapy has shortened the course of many complications which are not fatal but which may cause painful or harassing symptoms. There is, however, one very common manifestation of primary pulmonary tuberculosis which, in our hands, has not shown any striking response to antimicrobial therapy. We refer to endobronchial disease due to encroachment of the caseous bronchopulmonary or mediastinal lymph nodes on the bronchi.

Our early experience with specific therapy of this form of tuberculosis was reported with Daly and associates in 1952. The results of therapy were disappointing. There have been many reports such as those by Roberts and Blair, and Bentley in the British literature which also stress the lack of response of endobronchial disease to antimicrobial therapy. Boyd of Toronto reports good response to isoniazid treatment in children. The failure to respond to therapy was unexpected in our early cases in view of the rapid response of endobronchial disease in adults to antituberculous drugs.

A review of the pathogenesis of this form of tuberculosis in adults and children, however, readily explains the difference in response to specific therapy. Endobronchial disease in adults is due to the bathing of the mucous membrane lining a bronchus with sputum containing tubercle bacilli, usually derived from an adjacent cavity. The ulceration begins in the mucosa lining the bronchus and, if diagnosed early, heals rapidly under antimicrobial therapy. Moreover the source of the infection, as in the cavity, is simultaneously affected by specific therapy so that relapse, or reinfection of the bronchial mucosa, is prevented.

[1] This is a preliminary report. It is hoped that the investigation will be completed by the end of 1956.

[2] This study was aided by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service; and from Parke, Davis and Company, Detroit, Michigan.

[3] Chest Clinic of the Children's Medical Service, Bellevue Hospital, and Department of Pediatrics, New York University College of Medicine, New York, New York.

In children endobronchial disease is a result of the encroachment of lymph nodes on the bronchi. It is most frequently seen in the very young, in whom the bronchi are small and easily compressible. The nodes become fixed to the bronchial walls by inflammatory changes and the infection progresses through the walls of the bronchi to the mucosal lining. Bronchoscopic observation in the early stage of endobronchial disease may show fixation of the node to the bronchus. This may be followed by evidence of extrinsic pressure even when the mucosal lining is intact. Later in the course of the disease mucosal inflammation and edema may be present which may then be replaced by ulceration or granulation.

The granulation tissue may become exuberant and ultimately result in pedunculated granulomata or polyps which may persist for months and frequently recur if removed surgically. Occasionally a caseous node erodes the bronchial wall, causing a sinus through which caseous material is extruded. The local condition may clear completely, leaving an apparently normal bronchus, although the process of healing may take months. Less commonly, a stricture develops at the site of the endobronchial disease. Frequently during the course of endobronchial disease segmental or lobar shadows may be seen roentgenographically, presumably as a result of bronchial obstruction.

Endobronchial disease caused by the encroachment of nodes on bronchi has been recognized for many years and is frequently seen in children with primary tuberculosis. While it may cause distressing symptoms in the young child, it is an infrequent cause of death. During a seventeen-year period before the advent of streptomycin, one child on our ward died of suffocation caused by the extrusion of large masses of caseating tissue into the bronchi and trachea. Several children died of tuberculous pneumonia following massive bronchogenic spread due to bronchial erosion. In general, however, the immediate outcome of endobronchial disease was favorable. However, MacPherson, Kent, and Jones and many other observers have reported that residual damage frequently persists in the segment or lobe originally obstructed by endobronchial disease.

The results of specific antimicrobial therapy must therefore be appraised from two viewpoints: (*1*) Does specific therapy shorten the course of the disease and promote more rapid healing, and (*2*) does it prevent permanent bronchial and pulmonary changes?

* * *

During the years 1947–1953 the effect of specific therapy on endobronchial tuberculosis was studied intensively in our chest clinic. During this period bronchoscopy revealed definite endobronchial lesions in 147 patients with primary tuberculosis. Obviously this number does not include all patients who had endobronchial disease. Many children were not bronchoscoped and it is not possible to visualize every lesion by endoscopy.

Although the ward population during the years of study was about equally divided between boys and girls, there were 91 males and 57 females in the group of patients with endobronchitis. Children less than four years of age constituted about 65 per cent of our ward patients, but 84 per cent of the group with endobronchial disease were less than four years of age. This fact confirms our orig-

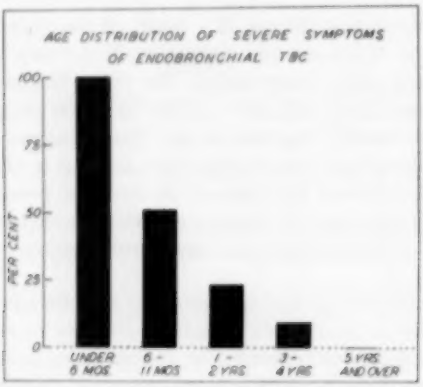



FIG. 1. Symptoms of endobronchial disease in relation to age.

TABLE 1

ROENTGENOGRAPHIC EVIDENCE OF BRONCHIAL OBSTRUCTION DUE TO ENDOBRONCHIAL DISEASE

| | | |
|---|---|---|
| Limited to 1 lobe | | 110 |
| Two or more lobes | | 20 |
| Entire lung | 2 | |
| Bilateral | 4 | |
| Emphysema only | | 5 |
| No evidence | | 12 |
| | | 147 |

inal impression that bronchial involvement occurs more frequently in the young child.

The most common presenting symptom was cough, but in the older group this was frequently absent and the diagnosis of endobronchial disease was suggested only by roentgenographic findings. Severe symptoms such as dyspnea, cyanosis, and marked spasmodic cough were seen most frequently in the young infant as shown in figure 1.

The roentgenographic evidence of bronchial obstruction due to endobronchial disease is shown in table 1.

The distribution of segmental obstruction is shown in figure 2. There are two sources of information from which one could hope to assess the value of antimicrobial therapy in endobronchial disease: the duration of roentgenographic evidence of segmental obstruction; and the character and duration of the disease as viewed by the bronchoscopist.

It is difficult to use duration of obstruction on the roentgenogram as an effective measure of response to therapy. A segmental or lobar shadow which disappears does not always mean re-expansion but may in some areas reflect a maximum shrinking of lung parenchyma. Moreover we have seen re-expansion of a segment on a roentgenogram associated with no change or a worsening of

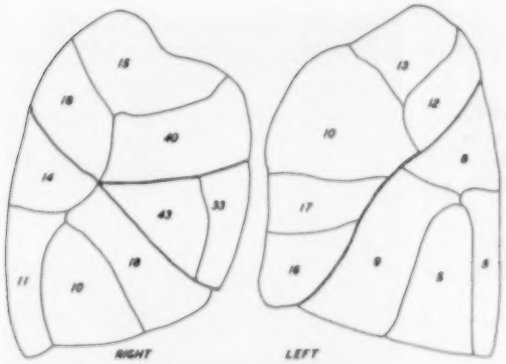



FIG. 2. Distribution of segmental obstruction, showing that segmental obstruction is more frequent in the right lung and most frequently seen in segment 3 of the right upper lung and segments 4 and 5 of the right middle lung.

TABLE 2

ENDOBRONCHIAL LESIONS SEEN ON BRONCHOSCOPY IN 147 PATIENTS

| | |
|---|---|
| External compression | 29 |
| Narrowing of lumen | 33 |
| Granulation tissue (Polyps—32) | 86 |
| Ulceration | 18 |
| Caseous material | 43 |
| Erosion of bronchial wall | 12 |

the degree of endobronchial disease. A more accurate method of following the course of endobronchial disease is by repeated bronchoscopies, and an attempt has been made to relate the bronchoscopic findings to the use of specific therapy.

There were many variations in treatment. During the early period of therapy with streptomycin, patients sometimes were treated for six weeks. Subsequently longer courses of treatment were advocated and some patients received six or more months of streptomycin along with Promizole or PAS. Still later isoniazid in combined therapy was given for a year. Moreover, some of the patients were being treated primarily for meningitis or other forms of tuberculosis of serious prognosis and all of these received intensive and prolonged therapy.

Because of the great variation in antimicrobial agents, in dosage and in duration of therapy, we have not attempted to analyze results according to various schemes of therapy but have included in the treated group all children who received six weeks or more of daily antimicrobial therapy with streptomycin or six months or more of isoniazid. One hundred and nine patients in our group of 147 received specific treatment.

The nature of the endobronchial disease in our group of patients may be seen in table 2.

TABLE 3

DURATION OF ACTIVE ENDOBRONCHIAL DISEASE BY BRONCHOSCOPY IN 96 PATIENTS

| | |
|---|---|
| <3 months | 6 |
| 3–5 months | 17 |
| 6–8 months | 21 |
| 9–11 months | 15 |
| >1 year | 26 |
| >2 years | 6 |
| >3 years | 5 |

Residual narrowing in 27

TABLE 4

BRONCHOGRAPHIC FINDINGS

| | |
|---|---|
| Total normal | 20 |
| Total abnormal | 34 |
| Crowded bronchi | 10 |
| Stenosis of bronchus | 6 |
| Cylindric bronchiectasis | 19 |
| Sacculated bronchiectasis | 5 |

One hundred and nine of our 147 patients have been followed by bronchoscopy until the bronchi appeared entirely normal or until an apparently irreversible end point was reached. In the other 38 cases bronchoscopic evidence of healing or of permanent damage has not been established as yet. The minimum time interval between a bronchoscopy showing definite tuberculous intrabronchial disease and a negative bronchoscopy was eight weeks. The maximum duration of active endobronchial disease in our series is more than three years.

Sometimes the intervals between bronchoscopies were unavoidably prolonged, making the timing of changes very difficult. For this reason in table 3 we have not included the findings in 13 children who had negative bronchoscopies recorded more than three months after a previous report of active disease. This leaves only 96 children, 80 of whom received antimicrobial therapy. For this reason any attempt at relating the duration of healing to therapy has been omitted from table 3.

Certainly we have no evidence here that antimicrobial therapy exerts a rapid beneficial effect on endobronchial disease. On the other hand, there is evidence in individual cases that endobronchial disease may progress for many months while the patient is on intensive therapy. I should like to illustrate this failure of response to therapy by citing a few cases:

R. C. was ten months old when he developed miliary tuberculosis followed by tuberculous meningitis. He was treated with 1 gm. of streptomycin daily for seven months and Promizole for three years. On admission, April, 1948, a segmental obstruction of the right middle lobe was visible roentgenographically, but he was not bronchoscoped until twenty months later after recovery from meningitis. At this time tuberculous granulation tissue

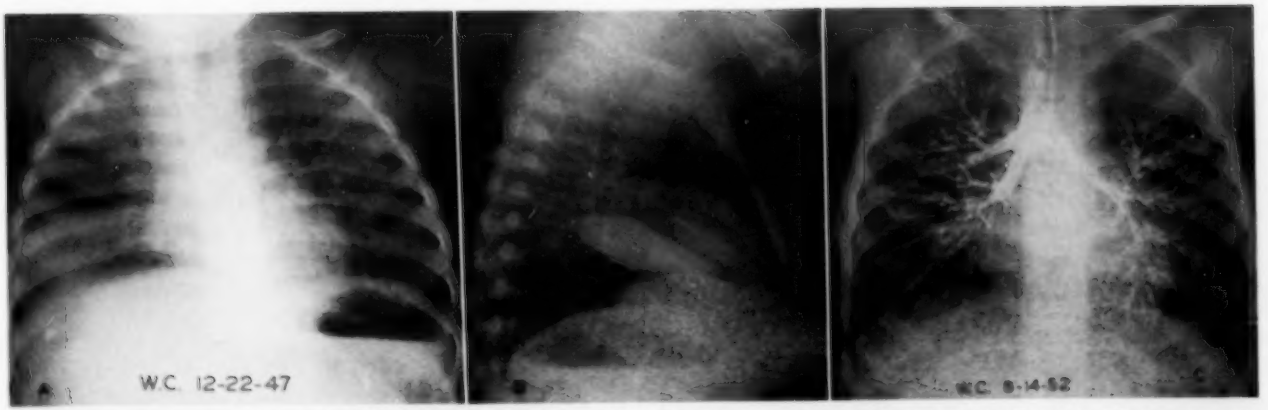



FIG. 3. W. C., seven months old, with severe symptoms of endobronchial disease and miliary tuberculosis. Treated with streptomycin and Promizole®, with good response of miliary disease; persistence of endobronchial disease for more than a year. *A*. Showing obstruction segment 3 of right lower lung, marked widening of mediastinum to right, and miliary tuberculosis. *B*. Lateral view showing emphysema behind involved segment. *C*. Showing normal bronchogram.

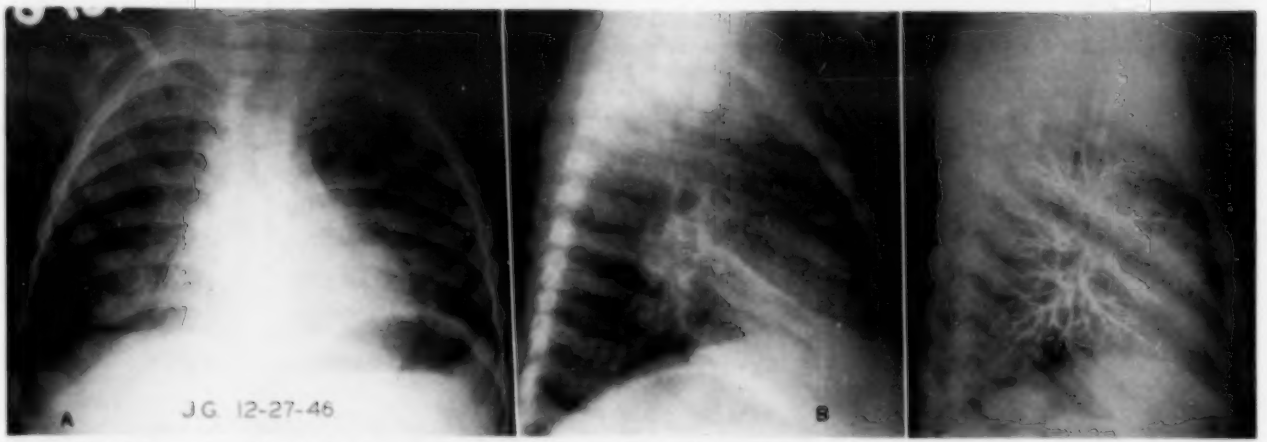



FIG. 4. J. G., one year old, with harsh persistent expiratory cough; no specific treatment. *A*. Showing prominent right root shadow and clouding medial half of right lower lung field. *B*. Lateral view interpreted as showing enlarged nodes and small obstructed right middle lung. *C*. Bronchogram showing crowding and downward displacement of right middle lung segmental bronchi.

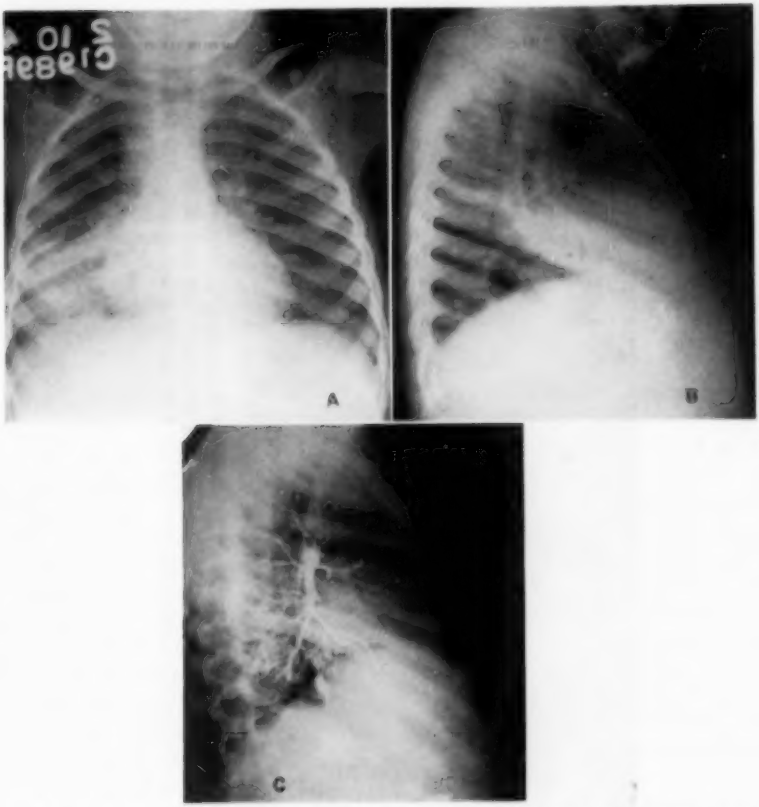



FIG. 5. E. S., three years old, with no symptoms of endobronchial disease. A granulomatous lesion on bronchoscopy healed in four months, resulting in stenosis, right middle lung bronchus. Patient was treated for three months with streptomycin and PAS. *A*. A roentgenogram interpreted as obstruction of entire right middle lung with widening of superior mediastinum to right. *B*. Lateral view. *C*. Bronchogram showing stenosis of right middle lung bronchus.

was present in the right middle lobe orifice which was still present four months later, fully two years after admission. Eventually the lesion healed leaving a stenosis of the right middle lobe orifice.

This case illustrates continuation or progression of endobronchial disease after prolonged specific therapy which cured meningitis and miliary tuberculosis.

L. W. was admitted in February, 1952, with progressive primary tuberculosis with cavitation, and treatment with isoniazid and Promizole was begun immediately. The patient made an excellent clinical response to therapy and no tubercle bacilli were cultured from gastric washings after two months of therapy. Bronchoscopy at this time showed granulation tissue in the orifices of the right middle lobe and right lower lobe, and polypoid

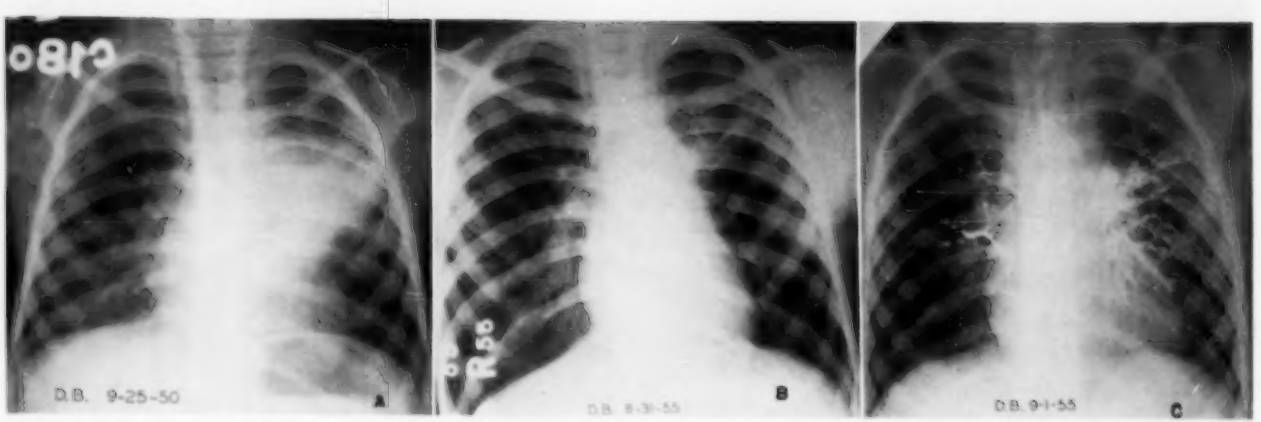



FIG. 6. D. B., three years old, with no symptoms of endobronchial involvement. Granulation and caseation tissue right lower lung bronchus persisted for four months; no further bronchoscopic follow-up observations. *A*. Large area of clouding in mid zone of left lung corresponding to segment 6 of left lower lung. *B*. Roentgenogram obtained five years later, showing residual streaking and clouding. *C*. Bronchogram showing saccular bronchiectasis in segment 6 of left lower lung.

granulation tissue was still present six months later. In November, 1953, after being on continuous isoniazid therapy for twenty-one months, bronchoscopy still revealed extensive disease and caseous material was expressed from granulation tissue in the right middle lobe bronchus.

G. M. was an infant first admitted in September, 1952, with severe symptoms of endobronchial disease and roentgenographic evidence of obstruction of the right middle lobe and emphysema. He was treated with isoniazid and Promizole for a year with the addition of PAS for six months of this time. During the following year he continued to have attacks of respiratory difficulty, with wheezing, and eighteen months after his original admission developed an obstruction of the anterior basal segment of the right lower lobe. Granulation tissue was seen in the segmental orifice. More recently this child has been free from symptoms, but bronchoscopy still shows granulation tissue more than three years after the original diagnosis. He has just completed six months of therapy with cycloserine without visible effect on the endobronchial lesion.

* * *

Since antimicrobial therapy apparently does not shorten the course of endobronchial disease due to caseous nodes, it is important to know whether therapy prevents the permanent bronchial and pulmonary changes which were so frequently demonstrated in the pre-chemotherapy era.

There has been a marked decrease in the incidence of bronchiectasis following pneumonias in all pediatric services and this is undoubtedly due to the efficacy of antimicrobials. A similar reduction in bronchiectasis in segments obstructed by tuberculous endobronchial disease has not yet been observed. A careful follow-up by bronchography is necessary to demonstrate the true incidence of damage to bronchi and parenchyma following obstruction due to endobronchial disease. These children do not present the classical picture of suppurative bronchiectasis and may be entirely free from symptoms. Follow-up study of our series of 147 children is in progress and so far bronchograms have been done without selection on more than one-third of the patients, and the results are shown in table 4.

The accompanying roentgenograms (figures 3–6) illustrate some of these end results and also show the difficulty of evaluating therapy.

* * *

In conclusion, there seems to be no evidence in our group of children that antimicrobial therapy has a beneficial effect in hastening the resolution of endobronchial disease due to encroachment of caseous nodes on bronchi. Nor have we any evidence that the incidence of damaged parenchyma and bronchi has been diminished by the use of specific antituberculous therapy.

We are not saying that children with endobronchial disease should not be treated; only that there is no evidence of response to therapy.

A second study is already planned which may give a more definite answer since there will be fewer variables. Since 1953 all children with primary tuberculosis have been treated on a randomized basis; the treated patients have all received isoniazid, 5 mg. per kg., for a year. Many of these children have shown endobronchial disease on bronchoscopy, and ultimately we hope to be able to compare the course of endobronchial disease in the treated and control groups.

# EXPERIENCES WITH INTRA- AND EXTRALUMINAL BRONCHIAL TUBERCULOUS LESIONS

ROBERT H. HIGH[1] AND WALDO E. NELSON[1]

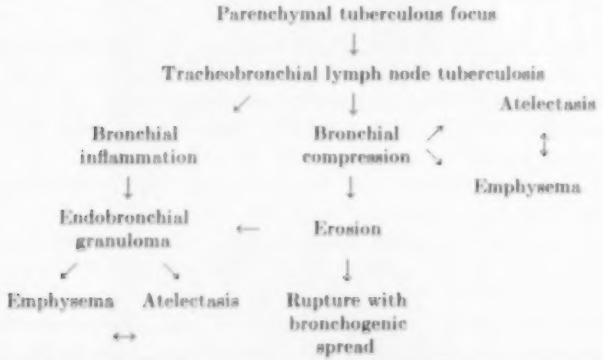
The progression of pulmonary tuberculosis from a focus in the parenchyma of the lungs to the regional lymph nodes has been extensively studied at postmortem examination in infants and children, and in animals by experimental techniques. The progress of the infection in the living patient can only be imperfectly studied by roentgenographic examination.

This method of examination is particularly useful in following changes in the pulmonary parenchyma. None of the above methods of investigation is entirely satisfactory for the observation of changes in the trachea and bronchi related to tuberculous processes. Such changes can best be observed during life by inspection of the trachea and bronchi by bronchoscopic examination.

Although the abnormalities in the trachea and bronchi secondary to pulmonary tuberculosis have been reviewed in a few excellent articles (1–4), there is a paucity of information related to the findings observed during bronchoscopy in infants and children with pulmonary tuberculosis. Such observations are helpful in understanding various pulmonary abnormalities, especially those related to obstructive atelectasis and to obstructive emphysema.

The former changes are sometimes designated as "epituberculosis" or a noninfectious exudative lesion, but in all probability are chiefly the result of rather abrupt obstruction of a lobar or segmental bronchus. Rapid clearing of pulmonary lesions is often related to the spontaneous regression of obstructive lesions.

A simplified schema showing how the bronchial changes secondary to parenchymal or lymph node tuberculosis produce various clinical patterns is shown below. This schema, like all generalizations, is not completely inclusive but is presented as an aid in the understanding of how certain clinical patterns observed in infants and children with intrathoracic tuberculosis are produced.




[1] Department of Pediatrics, Temple University School of Medicine; and St. Christopher's Hospital for Children, Philadelphia, Pennsylvania.

TABLE 1

DISTRIBUTION OF PATIENTS EXAMINED BY BRONCHOSCOPY ACCORDING TO TYPE OF LESION

| Lesion | Philadelphia General Hospital Northern Division | | St. Christopher's Hospital for Children | | Bronchoscopy | | |
|---|---|---|---|---|---|---|---|
| | Total | Bronchoscopy Number | Total | Bronchoscopy Number | Total | Number | Per Cent |
| Parenchymal | 42 | 18 | 8 | 6 | 50 | 24 | 48.0 |
| Hilar | 33 | 4 | 5 | 4 | 38 | 8 | 21.0 |
| Parenchymal and hilar | 27 | 14 | 22 | 21 | 49 | 35 | 71.4 |
| Other* | 7 | 0 | 0 | 0 | 7 | 0 | 0 |
| Total | 109 | 36 | 35 | 31 | 144 | 67 | 46.5 |

* Includes miliary tuberculosis and effusion.

This report is presented in the hope that the findings observed in a large number of bronchoscopic examinations will be helpful for a better understanding of the progression of pulmonary tuberculosis in infants and children.

## MATERIAL

This study is based on an analysis of the hospital course of 144 infants and children with pulmonary tuberculosis who were discharged from St. Christopher's Hospital for Children or the Northern Division of the Philadelphia General Hospital[2] between January 1, 1950, and September 30, 1955. Patients with various types of extrapulmonary tuberculosis, chiefly tuberculous meningitis, are not included in this study. Patients who merely showed calcified lesions in the pulmonary parenchyma or tracheobronchial lymph nodes are likewise excluded from this study.

In 67 of the 144 patients, bronchoscopic examination was performed on one or more occasions.[3] The observations made during the bronchoscopic examinations are reviewed separately and form the major portion of this report.

The bronchoscopic examinations were performed when the patients were in the fasting state. Younger infants received no premedication, whereas older infants and children received a barbiturate and sometimes an opiate as preoperative medication. Local anesthesia was occasionally applied to the mucosa of the hypopharynx and larynx. General anesthesia was never employed. Except for a very occasional instance of transient laryngospasm, there were no complications following bronchoscopy.

## RESULTS

The distribution of the 144 patients according to the major type of pulmonary disease is shown in table 1, as is the number of patients examined by bronchoscopy. It is to be noted that the patients at St. Christopher's Hospital for Children in general had somewhat more extensive disease. Furthermore, the percentage of those bronchoscoped in this group is greater than that of those at the Northern Division of Philadelphia General Hospital. Of the 144 patients,

[2] The chiefs of service were, successively, Dr. Louis Cohen, deceased, and Dr. Charles Heiken.

[3] Bronchoscopic examinations at St. Christopher's Hospital for Children were performed by Dr. Emily Van Loon and her associates; those at the Northern Division of Philadelphia General Hospital were performed by Dr. William Lell and his associates.

TABLE 2

RESULTS OF CULTURES FOR M. TUBERCULOSIS OBTAINED FROM 67 PATIENTS EXAMINED BY BRONCHOSCOPY ACCORDING TO RACE AND SEX

| Result | White | | | | Negro | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | | |
| | St. C | PGH | St. C | PGH | St. C | PGH | St. C | PGH | |
| Positive | 6 | 2 | 3 | 1 | 10 | 11 | 6 | 5 | 44 |
| Negative | 1 | 1 | 0 | 0 | 2 | 7 | 1 | 8 | 20 |
| No report | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 3 |

St. C = St. Christopher's Hospital for Children.
PGH = Philadelphia General Hospital.

67 (46.5 per cent) were bronchoscoped at least once. This latter group of 67 patients who were examined by bronchoscopy is reviewed in more detail.

The diagnosis of pulmonary tuberculosis was made only in those patients who had positive tuberculin reactions and abnormalities in the roentgenograms of their chests compatible with this disease. A history of contact with a known tuberculous patient was established in most instances. Bacteriologic cultures were obtained in all but 3 of the 67 patients with whom we are concerned in this report. The results of these cultures are presented in table 2. The cultures were positive for *M. tuberculosis* in 65 per cent of the group. The percentage of positive cultures was somewhat greater in the patients studied at St. Christopher's Hospital for Children than in those at Philadelphia General Hospital, Northern Division, being 86 and 54 per cent, respectively.

The distribution of patients examined by bronchoscopy according to their age, race, and sex is listed in table 3. It is apparent that most of the patients were in the younger age group. Infants accounted for 32 (approximately 45 per cent) of the total group, whereas children more than five years of age numbered 11 (approximately 16 per cent) of those bronchoscoped.

The high prevalence of Negro children, 51 of the total of 67 infants and children bronchoscoped, reflects the greater prevalence of tuberculosis in Negroes in the Philadelphia area. The slight preponderance of males is not considered significant.

The number of patients subjected to bronchoscopic examination is listed in table 4, according to their race, sex, and the hospital in which they were treated. A total of 148 bronchoscopic examinations was performed; once in each of 34 patients, whereas 33 infants and children were bronchoscoped on two or more occasions. The 33 patients who were examined on more than one occasion accounted for 114 of the total number of bronchoscopic examinations.

Almost all of the bronchoscopic examinations revealed some abnormality. Normal mucosa was observed in only 14 examinations (table 5). As will be noted in material presented subsequently, 10 of the patients with normal tracheobronchial mucosa had shown marked improvement in the abnormalities previously noted in the roentgenograms of their chests. Four of the patients did not have such evidence of improvement. Inflammation of the tracheobronchial

TABLE 3

DISTRIBUTION OF PATIENTS EXAMINED BY BRONCHOSCOPY ACCORDING TO AGE, RACE, AND SEX

| Age in Years | White | | | | Negro | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | | |
| | St. C | PGH | St. C | PGH | St. C | PGH | St. C | PGH | |
| 0–1 | 1 | 1 | 1 | 0 | 4 | 5 | 0 | 1 | 13 |
| 1–2 | 3 | 1 | 2 | 0 | 2 | 4 | 4 | 3 | 19 |
| 2–3 | 2 | 1 | 1 | 1 | 3 | 4 | 2 | 3 | 17 |
| 3–4 | 0 | 1 | 0 | 0 | 1 | 2 | 1 | 0 | 5 |
| 4–5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| 5–6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| 6–7 | 1 | 0 | 0 | 0 | 2 | 2 | 0 | 1 | 6 |
| 7–8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| 8–9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9–10 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| 10–11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Total | 7 | 4 | 4 | 1 | 12 | 18 | 8 | 13 | 67 |

St. C = St. Christopher's Hospital for Children.
PGH = Philadelphia General Hospital.

TABLE 4

NUMBER OF BRONCHOSCOPIC EXAMINATIONS ACCORDING TO RACE AND SEX

| Number of Examinations | White | | | | Negro | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | Male | | Female | | |
| | St. C | PGH | St. C | PGH | St. C | PGH | St. C | PGH | |
| 1 | 3 | 1 | 2 | 1 | 2 | 11 | 6 | 8 | 34 |
| 2 | 2 | 2 | 1 | 0 | 4 | 4 | 0 | 5 | 18 |
| 3 | 0 | 1 | 0 | 0 | 4 | 1 | 1 | 0 | 7 |
| 4 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 3 |
| 5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 10 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |

St. C = St. Christopher's Hospital for Children.
PGH = Philadelphia General Hospital.

mucosa was noted to be the only abnormality in 68 of the 148 examinations, but was observed in conjunction with other abnormalities in 59 additional instances.

The inflammatory changes were almost always more marked on the same side as the pulmonary lesions, but occasionally bilateral or even contralateral

TABLE 5

OBSERVATIONS DURING BRONCHOSCOPIC EXAMINATION

| Observations | St. Christopher's Hospital | Philadelphia General Hospital Northern Division | Total |
|---|---|---|---|
| Normal | 7 | 7 | 14 |
| Inflammation | 30 | 38 | 68 |
| Compression | 6 | 1 | 7 |
| Inflammation and compression | 39 | 6 | 45 |
| Inflammation and granuloma | 2 | 3 | 5 |
| Inflammation, compression, and granuloma | 7 | 2 | 9 |
| Total | 91 | 57 | 148 |

inflammatory changes were observed. The inflammatory changes consisted of increased redness of the mucosa with or without increased tracheobronchial secretions. The increased amount of secretion was commonly greatest near the lobar bronchi which supplied the area of parenchymal disease. Edema and thickening of the tracheobronchial mucosa were sometimes observed.

Tuberculous granulomas were observed in 8 patients at 14 (approximately 9 per cent) of the examinations. Removal of this material by endoscopic means was followed by rapid relief of obstructive atelectasis or obstructive emphysema in 4 of the 8 patients from whom such abnormal tissue was removed. Erosion of the trachea or bronchi by adjacent tuberculous lymph nodes was not noted in this group, although, as is well known, such erosion can occur. The absence of findings of such erosion in this series suggests that it is not common in infants and children with pulmonary tuberculosis.

The observations at bronchoscopy of each patient are correlated with the changes observed in the roentgenogram of his chest in tables 6 to 10. Special attention is directed to tables 7 to 10, in which the detailed observations of the repeated bronchoscopic examinations are shown. Perusal of these latter four tables indicates that improvement in the abnormalities noted by bronchoscopic examination generally tended to parallel decreased abnormalities in the chest roentgenograms. However, numerous exceptions were observed. In general, normal tracheobronchial mucosa was rarely noted if roentgenographic abnormalities were still present.

A detailed analysis of the antituberculous treatment administered is not presented. Such treatment was dictated by the types of pulmonary lesions observed in individual patients. Antibacterial treatment was employed in 17 of the 31 patients treated at St. Christopher's Hospital for Children, and in 11 of the 36 patients treated at Philadelphia General Hospital, Northern Division.

In addition, prolonged administration of oxygen was necessary in 4 patients who had severe obstructive emphysema. One child, *Case No. 3* (table 8) developed a tension pneumothorax on two occasions. During the first episode, a single aspiration was sufficient to provide relief, but the second episode of pneu-

TABLE 6

OBSERVATIONS DURING A SINGLE BRONCHOSCOPIC EXAMINATION

| Number | Race and Sex | Age Last Birthday | Culture for *M. Tuberculosis* | Bronchoscopic Observations | | | | Roentgenographic Changes from First Examinations |
|---|---|---|---|---|---|---|---|---|
| | | | | Normal | Inflammation | Compression | Granuloma | |
| 1 | N.F. | 2 | + | | X | | | Same |
| 2 | N.F. | 6 | − | | X | | | Same |
| 3 | N.M. | 1 | − | | X | | | Same |
| 4 | N.M. | 2 | + | X | | | | Same |
| 5 | N.F. | 1 | − | | X | | | Same |
| 6 | N.M. | 2 | + | | X | | | Improved |
| 7 | N.M. | 6 | − | | X | | | Improved |
| 8 | N.M. | 1 | + | | X | | | Same |
| 9* | N.M. | 3 | + | | X | | | Same |
| 10 | N.M. | 1 | − | | X | | | Same |
| 11 | N.F. | 11 | + | | | X | | Improved |
| 12 | N.M. | 6 | − | | X | | | Same |
| 13 | N.F. | 4 | − | | X | | | Same |
| 14 | W.M. | 3 | Not done | | X | | | Same |
| 15 | N.M. | 0 | + | | X | | | Improved |
| 16 | N.F. | 1 | − | | X | | | Same |
| 17 | N.F. | 2 | + | | X | | X | Improved |
| 18 | N.M. | 0 | + | | X | | X | Improved |
| 19 | W.F. | 2 | − | | X | | | Improved |
| 20 | N.F. | 7 | − | | X | | | Same |
| 21 | N.M. | 0 | + | | X | | | Same |
| 22 | W.F. | 1 | Not done | | X | X | | Same |
| 23 | N.F. | 2 | + | | X | X | | Same |
| 24 | N.F. | 2 | + | | X | | | Same |
| 25 | W.M. | 2 | + | | X | | | Same |
| 26 | W.M. | 6 | + | X | | | | Same |
| 27 | N.F. | 1 | + | | X | X | | Same |
| 28 | N.F. | 1 | + | | X | X | | Same |
| 29 | N.F. | 1 | Not done | | X | | | Same |
| 30 | W.M. | 1 | − | | | X | | Same |
| 31 | N.M. | 2 | + | | | X | | Same |
| 32 | W.F. | 2 | + | | X | | | Worse |
| 33 | N.F. | 3 | + | | X | | | Improved |
| 34 | N.M. | 3 | + | | | X | | Same |

* Died: anesthesia accident.

Note: Patients 1 to 21 were examined at Philadelphia General Hospital, Northern Division, and patients 22 to 34 were examined at St. Christopher's Hospital for Children.

mothorax was controlled only by prolonged, continuous intrapleural aspiration.

Bronchograms were performed on 2 patients. Only one was abnormal, and the surgical removal of the right middle and lower lobes was performed in one child, *Case No. 3* (table 10). This patient developed bronchostenosis and secondary bronchiectasis in the area previously involved by tuberculosis. His postoperative course and subsequent progress were without incident.

In the total group of 144 patients, there were 2 deaths. In neither instance

TABLE 7

OBSERVATIONS DURING TWO BRONCHOSCOPIC EXAMINATIONS

| Number | Race and Sex | Age Last Birthday | Culture for *M. Tuberculosis* | Bronchoscopic Observations | | | | Roentgenographic Changes from First Examinations |
|---|---|---|---|---|---|---|---|---|
| | | | | Normal | Inflammation | Compression | Granuloma | |
| 1 | W.M. | 2 | − | | X | | | Same |
| | | | | X | | | | Improved |
| 2 | W.M. | 0 | + | X | | | | Same |
| | | | | | X | | | Improved |
| 3 | N.F. | 0 | + | | X | | | Same |
| | | | | | X | | | Same |
| 4 | N.F. | 1 | + | | X | | | Same |
| | | | | X | | | | Improved |
| 5 | N.M. | 0 | − | | X | | | Same |
| | | | | | X | | | Improved |
| 6 | N.M. | 3 | − | | X | | | Improved |
| | | | | | X | | | Improved |
| 7 | N.F. | 2 | − | | X | | | Same |
| | | | | | X | | | Same |
| 8 | N.M. | 1 | − | | X | X | | Same |
| | | | | | X | X | | Improved |
| 9 | N.M. | 2 | + | | X | | | Same |
| | | | | | X | | | Improved |
| 10 | N.F. | 4 | − | | X | X | | Same |
| | | | | | X | X | | Improved |
| 11 | N.F. | 7 | + | | X | | X | Same |
| | | | | X | | | | Improved |
| 12 | N.M. | 0 | + | | X | | | Improved |
| | | | | | X | | | Improved |
| 13 | N.M. | 2 | + | | X | X | | Same |
| | | | | | X | | | Improved |
| 14 | W.M. | 2 | + | | X | X | | Same |
| | | | | | X | X | | Same |
| 15 | N.M. | 6 | + | | X | | | Same |
| | | | | | X | | | Worse |
| 16 | W.M. | 1 | + | | X | | | Same |
| | | | | | X | | | Improved |
| 17 | W.F. | 0 | + | | X | X | | Improved |
| | | | | | X | | | Improved |
| 18 | N.M. | 6 | − | | X | | | Same |
| | | | | | X | | | Same |

Note: Patients 1 to 11 were examined at Philadelphia General Hospital, Northern Division, and patients 12 to 18 were examined at St. Christopher's Hospital for Children.

did the death result from the tuberculous process. In one of the 67 patients who were examined by bronchoscopy, death followed the injection of local anesthesia in preparation for a thoracentesis (*See Case No. 9*, table 6). One patient in the group of 77 patients who were not bronchoscoped died of cardiac arrest during an operation for bilateral inguinal hernias. He had had complete clearing of miliary tuberculosis.

TABLE 8

OBSERVATIONS DURING THREE BRONCHOSCOPIC EXAMINATIONS

| Number | Race and Sex | Age Last Birthday | Culture for *M. Tuberculosis* | Bronchoscopic Observations | | | | Roentgenographic Changes from Admission or Preceding Examinations |
|---|---|---|---|---|---|---|---|---|
| | | | | Normal | Inflammation | Compression | Granuloma | |
| 1 | W.M. | 1 | + | | X<br>X<br>X | X<br>X | | Same<br>Same<br>Same |
| 2 | N.M. | 9 | + | | X<br>X<br>X | | | Same<br>Improved<br>Improved |
| 3 | N.M. | 1 | + | | X<br>X<br>X | X<br>X<br>X | X<br>X | Same<br>Same<br>Same |
| 4 | N.M. | 0 | + | <br><br>X | X<br>X | X | | Improved<br>Improved<br>Improved |
| 5 | N.F. | 5 | − | <br><br>X | X<br>X | | | Same<br>Improved<br>Improved |
| 6 | N.M. | 0 | + | <br><br>X | X<br>X | X<br>X | | Same<br>Same<br>Improved |
| 7 | N.M. | 1 | + | <br><br>X | X<br>X | <br>X | | Same<br>Same<br>Same |

Note: Patients 1 and 2 were examined at Philadelphia General Hospital, Northern Division, and patients 3 to 7 were examined at St. Christopher's Hospital for Children.

TABLE 9

OBSERVATIONS DURING FOUR BRONCHOSCOPIC EXAMINATIONS

| Number | Race and Sex | Age Last Birthday | Culture for *M. Tuberculosis* | Bronchoscopic Observations | | | | Roentgenographic Changes from Admission or Preceding Examinations |
|---|---|---|---|---|---|---|---|---|
| | | | | Normal | Inflammation | Compression | Granuloma | |
| 1 | N.M. | 0 | + | <br><br><br>X | X<br>X<br>X | | | Same<br>Same<br>Same<br>Improved |
| 2 | N.M. | 2 | + | <br><br><br>X | X<br>X<br>X | X<br>X | X<br>X | Same<br>Same<br>Improved<br>Improved |
| 3 | W.F. | 1 | + | | X<br>X<br>X<br>X | X<br>X<br>X | | Same<br>Same<br>Improved<br>Improved |

Note: Patients 1 and 2 were examined at Philadelphia General Hospital, Northern Division, and patient 3 at St. Christopher's Hospital for Children.

TABLE 10

OBSERVATIONS DURING FIVE OR MORE BRONCHOSCOPIC EXAMINATIONS

| Number | Race and Sex | Age Last Birthday | Culture for *M. Tuberculosis* | Bronchoscopic Observations | | | | Roentgenographic Changes from Admission or Preceding Examinations |
|---|---|---|---|---|---|---|---|---|
| | | | | Normal | Inflammation | Compression | Granuloma | |
| 1 | N.M. | 0 | − | | X | X | | Worse |
| | | | | | X | X | X | Same |
| | | | | | X | X | | Improved |
| | | | | | X | X | X | Improved |
| | | | | | X | X | | Improved |
| 2 | N.F. | 1 | + | | X | X | | Same |
| | | | | | X | X | | Same |
| | | | | | X | X | | Improved |
| | | | | | X | X | X | Same |
| | | | | | X | X | | Same |
| | | | | | X | | | Improved |
| | | | | X | | | | Improved |
| 3 | W.M. | 1 | + | | X | X | | Same |
| | | | | | X | | | Same |
| | | | | | X | | | Improved |
| | | | | | X | | | Improved |
| | | | | | X | | | Worse |
| | | | | | X | | | Same |
| | | | | | X | X | | Same |
| | | | | | X | X | | Same |
| | | | | | X | X | | Same* |
| 4 | W.M. | 0 | + | | X | X | | Same |
| | | | | | X | X | | Worse |
| | | | | | X | X | | Same |
| | | | | | X | X | | Same |
| | | | | | | X | | Worse |
| | | | | | | X | | Improved |
| | | | | | X | X | | Same |
| | | | | | X | X | | Improved |
| | | | | | X | | | Improved |
| | | | | | X | | | Improved |
| 5 | N.M. | 2 | + | | X | X | | Same |
| | | | | | X | X | | Worse |
| | | | | | X | X | | Same |
| | | | | | X | X | | Worse |
| | | | | | X | X | | Same |
| | | | | | X | X | X | Improved |
| | | | | | X | X | X | Same |
| | | | | | X | X | | Improved |
| | | | | | X | | | Improved |
| | | | | | X | | | Same |
| | | | | | X | | X | Worse |
| | | | | | X | | X | Improved |
| | | | | | X | | | Worse |
| | | | | X | | | | Normal |

* Right middle and right lower lobe lobectomy at this point.

Note: All patients were examined at St. Christopher's Hospital for Children.

Systematic observations have not been possible on all the patients after their discharge from the two hospitals. Approximately half of the patients have been followed, and none has shown any complications to this date.

## DISCUSSION

The present study reveals that almost all of the patients examined by bronchoscopy had some abnormality in the trachea and/or bronchi. Inflammatory changes in the mucosa were most frequently observed. Although there is a possibility that some of the inflammatory changes resulted from secondary bacterial infections, it would seem much more likely that such changes were the result of the tuberculous infection.

Extraluminal compression associated with inflammatory changes in the mucosa was the second most common abnormality. In a number of instances, extraluminal compression was noted in the lobar bronchi supplying lobes which were atelectatic. Endobronchial granulations were observed relatively infrequently; they were noted in only 14 of the 148 examinations. However, it seemed important that these be discovered because their removal was at times followed by the relief of obstructive atelectasis or obstructive emphysema. Symptomatically patients with obstructive emphysema benefited to a greater extent than did those with obstructive atelectasis.

Although relatively few patients had immediate improvement following bronchoscopy, this procedure is a useful one. Bronchoscopy offers an opportunity to study the course of pulmonary tuberculosis in infants and children. Bronchial secretions provide the best specimens for bacteriologic study for *M. tuberculosis* as well as for other pulmonary pathogens. The presence of extraluminal compression explains many instances of persistent lobar atelectasis.

The occasional occurrence of bronchostenosis with bronchiectasis distal to the area of stenosis can be recognized early. Patients who have obstructive emphysema are especially in need of bronchoscopy, since some of these patients have endobronchial granulomas. The removal of such granulomas may be followed by prompt or even immediate relief of the obstructive symptoms.

Occasionally erosion of the bronchial wall occurs secondarily to inflammatory changes in adherent, adjacent lymph nodes; fortunately this complication was not observed in this series. Under such circumstances, bronchial aspiration may remove caseous material which could otherwise produce diffuse bronchogenic spread of the tuberculous process.

## SUMMARY

The present study reports the results observed in 67 infants and children with pulmonary tuberculosis who were examined bronchoscopically. In these patients, 148 bronchoscopic examinations were performed.

The findings in the 67 patients who were examined bronchoscopically are analyzed in detail, according to age, race, sex, number of bronchoscopies, and changes observed at serial bronchoscopic examinations. The bronchoscopic observations were correlated with the roentgenograms.

Of the abnormalities observed by bronchoscopic examinations, inflammation of the mucosa of the tracheobronchial tree was most common. It was observed as the only change in 68 instances. In addition, similar inflammatory changes were observed in association with other abnormalities in 59 additional examinations.

Endobronchial granulomas were noted on 14 occasions, or in approximately 9 per cent of the examinations. The removal of such granulation tissue resulted in prompt relief of obstructive manifestations in 4 of 8 patients.

Bronchoscopic examination is a valuable diagnostic, therapeutic, and investigative measure in infants and children with pulmonary tuberculosis.

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# BRONCHOSCOPY IN PRIMARY TUBERCULOSIS OF CHILDHOOD

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## INTRODUCTION

In 1950, the experiences of the pediatric division of the National Jewish Hospital with the bronchial involvement of primary tuberculosis of childhood were reported (1). Tuberculous tracheobronchitis in adults had been extensively studied bronchoscopically in this country, but we were impressed by the paucity of reports by American authors in reference to this condition in children. This discrepancy was explained in part by the reluctance of pediatricians to subject infants and young children to the "rigors and dangers still thought to be associated with bronchoscopic examination" (1). Another cause was the benign and asymptomatic state of many tuberculous children with mediastinal lymph node involvement. The frequency and hazards of bronchial perforation by mediastinal nodes had not been sufficiently stressed in the American literature. Finally, we expressed the opinion that bronchoscopic examination of tuberculous children had not been performed with "sufficiently adequate anesthesia to permit accurate observation" (1).

In the discussion that followed the presentation of our report, a seasoned contributor to the bronchoscopic literature of adult tuberculous tracheobronchitis asserted that "American physicians are more considerate of their children as patients; and, after all, treatment with a bronchoscope and laryngoscope are not playtime activities for these youngsters" (2). This approach apparently has not prevailed because many excellent bronchoscopic studies on tuberculous children have emanated from American sources since that time (3–5).

In our experience we noticed the primary compression of the bronchi which preceded perforation. In some cases, we were able to follow the sequence of events by serial bronchoscopy and to compare it with the pathologic development that occurs when tuberculous cervical nodes are ultimately extruded through the skin. We found bronchial perforation in almost half of the cases bronchoscoped—in good agreement with that reported by others.

Post-mortem investigations by Rich (6) plus the contributions of Schwartz (7) have emphasized this rather startling concept.

In spite of apparent disagreement by some (8), we believed that the roentgenographic shadows of so-called "epituberculosis" usually were the result of compression or obstruction of bronchi by the involved nodes. The resulting obstructive emphysema or atelectasis depended on the type of bronchial valvular mechanism. It was obvious that superimposed infiltration, bronchogenic spread, and pneumonitis could complicate these shadows. How much of the roentgenographic shadow complex could be attributed to these factors and how much to

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the obstructive effect was difficult to evaluate in any particular case. Aspirated foreign bodies which occur commonly in childhood and produce similar valvular effects in the bronchi present problems in differential diagnosis which have to be settled by bronchoscopy.

The discrepancies were impressive between the apparent parenchymal involvement in some cases when compared with the benign clinical course. The pattern of obstructive emphysema—more certainly diagnosed if roentgenograms were made both in inspiration and expiration—occurred less frequently than the wedge-shaped densities of atelectasis, but, we believed, was equally significant.

There were some cases of middle lobe syndrome and other types of "permanent atelectasis" with or without bronchiectasis. These we interpreted as examples of the late sequelae of primary infection.

Recent reports (3–5, 9, 10) show an increased awareness of this problem. In relation to bronchoscopy, many questions deserve consideration and it is with these that we will concern ourselves.

The bronchoscope has wrought tremendous changes in our concepts of pulmonary pathology. Its use even in the adult with pulmonary tuberculosis, at first, was restricted because of fear of spreading the disease (11). With increased awareness of its importance, bronchoscopy evolved into an almost routine procedure in many institutions. The knowledge thus gained has revealed the tremendous importance of the bronchogenic overlay in adult pulmonary tuberculosis.

Bronchoscopic examinations in adults were successfully performed with local anesthesia and with scarcely any risk. The pathologic changes observed in the bronchi of the living were not identical with those that had been described post mortem because terminal changes often complicated the picture. Similarly, autopsy findings in childhood tuberculosis are no true indication of the developing panorama of bronchial pathology which the bronchoscope can reveal in the living.

It should be enlightening to compare the findings in adults and children. The lesions in the bronchi of adults spread from the adjacent lung parenchyma by sputum, lymphatic or hematogenous routes, or by contiguous submucosal invasion. First, isolated tubercles are formed in the submucosa which are then followed by more extensive hyperplastic changes in larger areas. Often mucosal disorganization develops producing ulcerations and granulations which characteristically encircle the entire bronchial circumference. The isolated ulcer is more often found post mortem.

Occasionally in adults another type of involvement is observed as the result of perforation of the bronchial wall by necrotizing, mediastinal nodes. It is characterized by (a) the presence of caseous masses; (b) granulations at the site of perforation, showing little tendency to spread; (c) the prolonged period required for the necrotic lymph node contents to be expelled; (d) the lack of benefit from administration of antimicrobial drugs; and (e) roentgenographic shadows which as a rule demonstrate a minimum of parenchymal pulmonary involvement but reveal unusually large hilar nodes.

When streptomycin was introduced, it proved to be very effective in the control of the common type of adult tracheobronchitis, but had little effect on the lymph node perforating variety. The inference was that a more or less fixed time interval was required for the caseous nodes completely to extrude themselves. Streptomycin apparently did not hasten this process—although conceivably it helped to reduce the tuberculous reaction in the area around the lymph node. Another characteristic of the lymph node type was the scarcely noticeable stenosis that followed healing.

It was an enigma that, in the course of our own bronchoscopic experiences, no known case of bronchial breakthrough of the endothoracic lymph nodes in a child had been diagnosed, whereas the foreign literature abounded in such reports.

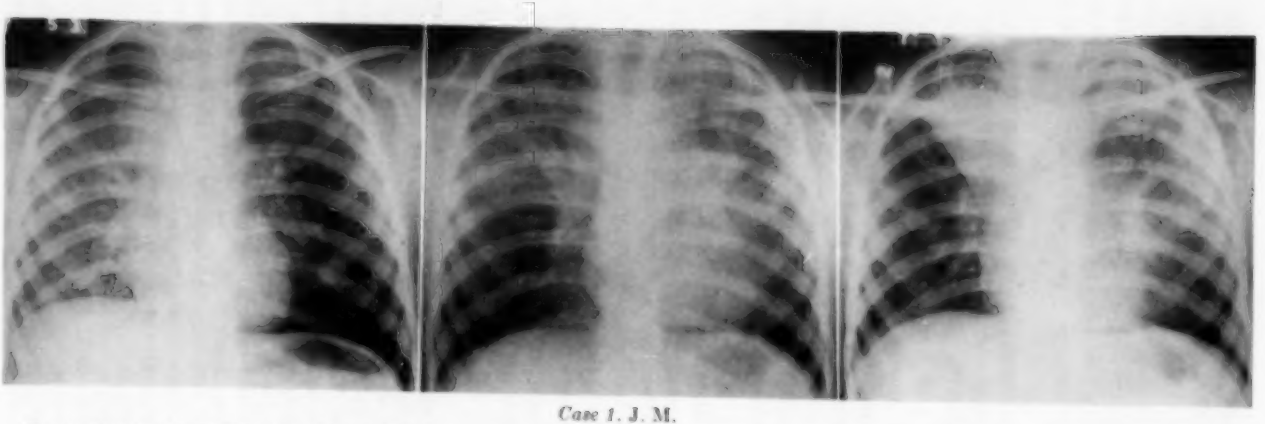
In 1948 a child of nineteen months in whom a foreign body was suspected because of wheezing, cough, and signs of partial atelectasis in the right lung field was referred for routine bronchoscopy. Instead of a foreign body, caseous masses were seen and removed from the right main stem bronchus. Tubercle bacilli were found in the specimen and the diagnosis of tuberculosis of the mediastinal lymph nodes with perforation was established. This aroused an immediate interest in the problem.

Over the ensuing years we have adopted liberal indications for bronchoscopy in children. Our patients have ranged from seven months to fourteen years of age. We have performed one hundred and seventy-two childhood bronchoscopies in 80 patients. We have not hesitated to repeat the procedure when indicated. Two patients were each serially bronchoscoped seven times. Among 52 cases showing definite suggestive clinical and roentgenographic signs, positive bronchoscopic findings could be demonstrated in 41; among the latter, evidence of perforation was demonstrated in 46 per cent.

### *Case Reports*

*Case 1.* J. M., a five-year-old Navajo girl, was admitted to the hospital on March 24, 1953, with active primary tuberculosis found by routine survey of family contacts following the diagnosis of tuberculosis in her mother (figure 1). She was a chronically ill, malnourished American Indian female child with positive physical findings limited to the chest. The right chest was essentially normal. The left chest did not expand well and the percussion note over the upper left chest was dull. In this same area, breath sounds were absent. She was treated with streptomycin (0.5 gm. daily to 0.5 gm. every third day) and isoniazid (75 mg. to 125 mg. daily). Bronchoscopy on April 2, 1953, revealed a thickened, flattened carina, and the left main stem and intermediate bronchi were partially obstructed by large amounts of caseous necrotic debris (figure 2). This material was aspirated, and acid-fast bacilli were found on microscopy. Following this aspiration, the physical findings shifted to involve the right upper chest, while the area occupied by the left upper lobe began to aerate. At that time signs of obstructive emphysema were obtained over the entire left chest (figure 3). Bronchoscopy on April 16, 1953, revealed both right upper and right middle lobe bronchial orifices obstructed by caseous necrotic debris (figure 4). At that time the left upper and intermediate bronchi were similarly obstructed. This material was aspirated and the process stabilized thereafter.

Bronchoscopy on May 26, 1953, revealed a granuloma on the wall of the left main stem bronchus at the junction of the anterior and lateral aspects. Finally, bronchoscopy revealed

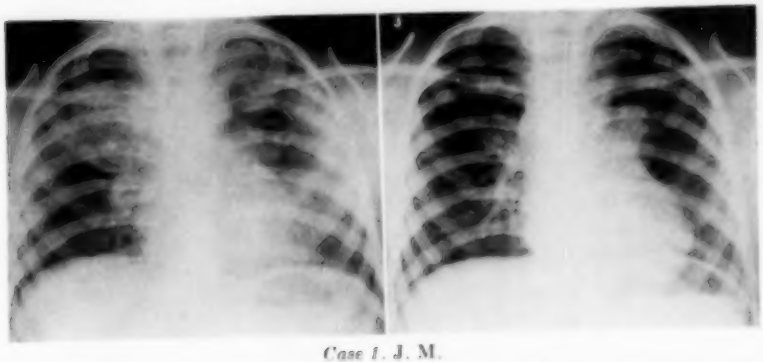


*Case 1.* J. M.

FIG. 1 (Left), March 27, 1953. The admission roentgenogram of a five-year-old Navajo Indian girl with extensive bilateral lesions, greater on the right, and with extensive involvement of the right hilar region.

FIG. 2 (Center), April 2, 1953. Roentgenogram obtained six days later; physical signs had shifted to the left side. Bronchoscopy immediately following this roentgenogram revealed thickened, flattened carina, and caseous necrotic debris partially obstructing the left main stem and intermediate bronchi. Much soft caseous material was removed.

FIG. 3 (Right), April 7, 1953. Roentgenogram obtained five days after figure 2; physical signs indicated left-sided obstructive emphysema; the right upper chest was dull to percussion, and breath sounds were absent. Fluoroscopy confirmed this.



*Case 1.* J. M.

FIG. 4 (Left), April 16, 1953. Bronchoscopy immediately following this roentgenogram revealed large amounts of caseous necrotic debris obstructing the right upper lobe and right middle lobe bronchial orifices. Much soft caseous material was removed.

FIG. 5 (Right), June 8, 1954. Final bronchoscopy revealed chronic granulomatous stenosis of the orifice of the left upper lobe bronchus. Roentgenograms were stable for six months at this time.

a chronic granulomatous stenosis of the left upper lobe bronchial orifice (figure 5). After this the process was considered to be inactive and the patient was discharged to continue isoniazid alone for three months more at her home on the Navajo reservation.

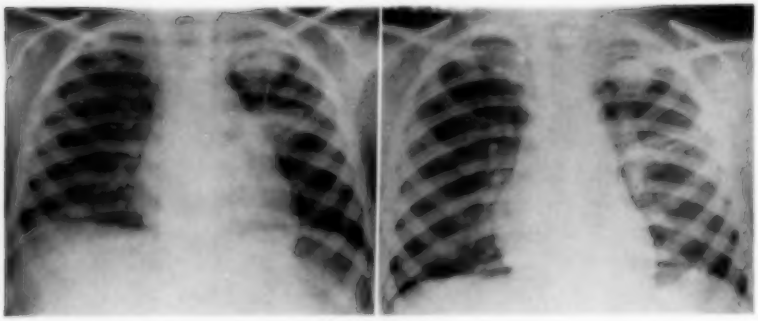
*Comment:* This patient displayed rapidly changing physical signs of both obstructive emphysema and atelectasis. In general, the caseous material aspirated at bronchoscopy was nearly completely liquefied. However, it was so copious in amount that it was believed strongly that this form of therapy was justified.

*Case 2.* W. L., a four-year-old Negro boy, was admitted to the hospital on May 15, 1951, with active primary tuberculosis (figure 6). At the age of six months he was diagnosed as having juvenile asthma. At three and one-half years he had had pneumonia, but persisting signs and symptoms plus a positive tuberculin skin reaction eventually led to the diagnosis of tuberculosis. He was bronchoscoped on March 12, 1951. An area of granulation in left upper lobe bronchus was seen. He was transferred to this hospital after three months and was treated with streptomycin (0.5 gm. every third day) and para-aminosalicylic acid (PAS) (3 gm. daily) (figure 6).

Bronchoscopy on July 30, 1951, revealed a small whitish area on the anterolateral surface of the left main stem bronchus, probably representing an area of perforation. Then on January 28, 1952, a friable granulation tissue papilla was seen extending out of the posterior wall of the left upper lobe bronchus. Three months later, bronchoscopy failed to reveal any gross lesions (figure 7). He did well, and was discharged to his home with a fully stabilized process on May 7, 1952.

*Comment:* This case is illustrative of the ordinary course of bronchial perforation, formation of granulation tissue, and healing, leaving no bronchoscopically visible residue.

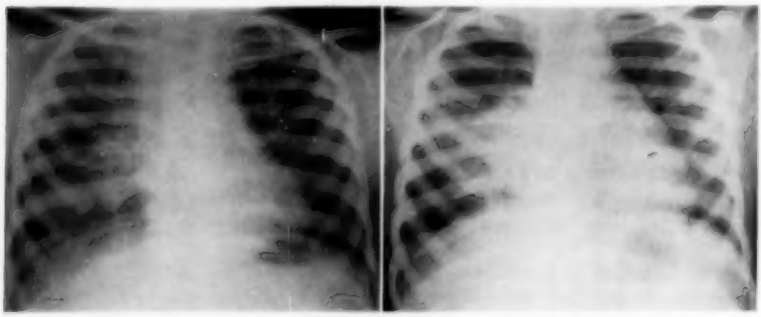
*Case 3.* W. J. C., a four-and-one-half-year-old Negro boy, was admitted to the hospital on June 15, 1954, with active primary tuberculosis (figure 8). He was treated with streptomycin



*Case 2.* W. L.

FIG. 6 (Left), May 22, 1951. Roentgenographic appearance of left lung lesions of four-year-old Negro boy following transfer to this hospital. Bronchoscopy revealed whitish area on the anterolateral surface of the left main stem bronchus.

FIG. 7 (Right), April 25, 1952. Bronchoscopy failed to reveal any gross lesions; no physical signs or symptoms. Roentgenographic findings were stable.



*Case 3.* W. J. C.

FIG. 8 (Left), June 15, 1954. The admission roentgenogram of four-and-a-half-year-old Negro boy revealed right middle lobe atelectasis. Bronchoscopy revealed granuloma at right middle lobe bronchial orifice and much caseous necrotic debris in the right lower lobe bronchus. Much soft caseous material was removed.

FIG. 9 (Right), July 9, 1955. Final bronchoscopy revealed that the granuloma remained but the right middle lobe bronchial orifice could not be visualized. At surgery on July 20, 1955, the bronchus to the anterior segment of the right upper lobe and the right middle lobe bronchus were found to be completely occluded. Segmental resection of the atelectatic anterior segment of the right upper lobe was carried out, as well as right middle lobe lobectomy. Convalescence was uneventful.

(0.5 gm. every third day) and isoniazid (125 mg. daily). Bronchoscopy on November 10, 1954, revealed a granuloma at the orifice of the right middle lobe bronchus and considerable caseous necrotic debris obstructing the right lower lobe bronchus.

The aspirated material showed acid-fast bacilli on microscopy. Bronchoscopy on May 10, 1955, revealed the granuloma and a caseous plug, which was aspirated. Final bronchoscopy still showed the granuloma, but the right middle lobe orifice could not be visu-

alized (figure 9). On July 20, 1955, the patient was subjected to segmental resection of the anterior segment of the right upper lobe, and right middle lobectomy. He withstood the procedure well, convalesced rapidly, and was discharged on September 13, 1955.

*Comment:* This case demonstrates failure of bronchoscopic treatment to prevent progressive stenosis which resulted in permanent fibrosis and destruction of the right middle lobe and anterior segment of the right upper lobe.

### BRONCHOSCOPIC FINDINGS

During bronchoscopy we look for the following pathologic changes:

*Widening of carina:* The sharp spur dividing the left from the right main bronchus becomes widened and rounded. This change in contour is in part due to the spreading effect of the enlarged lymph nodes and in part to the localized edema resulting from the contiguous inflammatory reaction.

*Inward displacement of tracheal wall:* The paratracheal nodes may indent and perforate the tracheal wall, although because of the greater rigidity of its rings this occurs less frequently than in the bronchi.

*Localized hyperemia:* Near the area of lymph node pressure the adjacent mucosa takes on a dusky hue. This is best evaluated if seen at some distance from the lesion before the bronchoscopic tube has passed over it. It must be emphasized that, in the bronchi of infants, even minimal contact with the bronchoscope results in mucosal congestion. Similarly, unless anesthesia is adequate to the point of suppressing the cough reflex, hyperemic mucosal changes which are seen should not necessarily be attributed to underlying pathology. Under such circumstances, bronchoscopy must be repeated at a later date. In the face of these disturbing factors, only the more advanced pathologic states can be judiciously evaluated.

*Increase of secretions:* Pooling of secretions in a stem bronchus should alert the bronchoscopist to look for pathology on that side. In the early stages the secretions may be mucoid. Later they become purulent and, when mixed with the contents of disintegrating nodes, assume a more gelatinous character. Since the tussive reflex is often impaired, secretions may stagnate. This may add to the obstructive factors and often accounts for the varying patterns seen in serial roentgenograms (*see* figures 1–5).

*Compression of bronchus:* The thin-walled bronchi of small children are easily compressed by extrinsic pressure. The bronchoscopic evolution of this externally produced narrowing requires careful observation and interpretation. It is well to remember that bronchi contract and dilate rhythmically, and these movements may be exaggerated by cough, spasm, and the irritation of the bronchoscope.

The proximally light bronchoscope affords the "bright light" over-all view which delineates relationships not brought out by distal lighting. When the region of the carina is passed, the entire stem bronchus together with its branches can be visualized in relation to each other. When the bronchoscopic tube passes through an externally compressed bronchus, it encounters minimal resistance; the lumen successively opens in front of it. On extraction of the tube, the lumen again collapses. This is in contrast to the sensation of tightness and difficult ad-

vance offered by the fibrous, stenosed bronchus whose lumen remains practically fixed.

*Perforation of bronchus:* The mucosa at the site of imminent breakthrough becomes thinned, glistening, and whitish, and has the appearance of a furuncle that is pointing or a tympanic membrane that is about to perforate.

With the use of strong bronchoscopic suction, this thinned-out mucosal area may be encouraged to rupture, permitting necrotic masses to be aspirated. There can be no serious objection to this maneuver in that it only aids nature in ridding the mediastinum of its necrotic foreign contents. An occasional fatality, the result of impaction in the glottis of a large caseous mass, may thus be averted. Instances of this kind have been reported in recent literature (12–16).

Bronchoscopic visualization of the site of perforation reveals that it may vary from pinhead size to several millimeters in diameter; multiple sites of perforation may also be seen. Granulations frequently develop in this region and may be sessile or pedunculated.

The time that it takes for the necrotic lymph node contents to be completely extruded varies from months to more than a year. We believe that treatment with use of the bronchoscope significantly shortened this period.

*Healed bronchus:* When healing finally occurs, surprisingly minor degrees of stenosis, or even no perceptible narrowing, may be demonstrated. This, of course, is true only if the process remains localized to the immediate site of rupture. Fortunately, this is the usual outcome. In any event, it is worth re-emphasizing that this lack of stricture formation is in sharp contrast to the crippling strictures which occurred in many cases of adult bronchial involvement, especially in the pre-chemotherapy era.

*Anesthesia and technical consideration:* We have found ether anesthesia safe, useful, and productive of the required relaxation. We are aware that ether in the adult tuberculous patient has been considered for a long time to be too dangerous. In children this objection apparently is not valid as we, and others who have used it, have had no reason for regret.

Daly and associates (3) introduced the use of Surital® combined with local procaine to anesthetize their children. Görgényi-Göttche and Kassay (17) found chloral hydrate useful in some of their cases.

Shane and Ashman (18) deprecate the "fluctuant" character of ether anesthesia. They advocate succinylcholine and hexobarbital preliminary to insufflating a mixture of $N_2O$-oxygen-cyclopropane through the side arm of the bronchoscope. If they cannot enter the child's larynx they resort to vinyl ether and open-drop ether before insufflating the above mixture. In our opinion, this is a needlessly complicated method as compared with time-proved ether anesthesia.

Experience has taught that it is wiser first to expose the larynx of infants with a laryngoscope before inserting the bronchoscopic tube. Conversely, in adults we have generally found preliminary laryngoscopy to be a decided technical disadvantage.

Adequate relaxation of the cords so that the bronchoscope can pass through an open glottis assures a minimum of subglottic reaction or none postoperatively.

Ether anesthesia deep enough to avoid the vagovagal reflex when the lower end of the trachea is reached will spare both the anesthetist and the operator some anxious moments.

When anesthesia becomes light for some reason, it is best to desist for a moment until further insufflation of ether vapor produces complete relaxation. In this way only can accurate evaluation of the tiny bronchial lumina of infants and young children be adequately obtained. Once excessive coughing and bronchial squeezing have set in, the best opportunity for accurate interpretation has been lost.

Permitting untrained residents to have the first look is an unrealistic approach when working in the delicate and confined bronchi of children.

Infant bronchoscopy need not be rushed. The reported findings of those who hasten to terminate their examination will not prove trustworthy.

*Reactions to bronchoscopy in children:* Post-bronchoscopic subglottic edema may occur when oversized tubes are used or when the bronchoscope is forced past a glottis which is in a state of spasm.

It is well to inspect the uninvolved side first. We found in our patients very little rise in temperature or clinical or roentgenographic signs of spread following this practice.

*Indication and contraindication for bronchoscopy:* All children with a positive Mantoux reaction who have wheezing, bibonal, ringing or brassy cough, dyspnea, or unexplained febrile course should be bronchoscoped. When roentgenographic shadows or physical examination suggest segmental, lobar, or whole lung atelectasis, bronchoscopy becomes imperative. Demonstration of obstructive emphysema also points to its need, as does the shadow depicting the middle lobe syndrome.

The Holzknecht-Jakobson sign—shifting of the mediastinal organs during deep inspiration to the side where bronchial stenosis exists—has been found to be a useful indication for bronchoscopy "even if it is the only sign of bronchial stenosis." This sign was reported positive in fourteen of fifteen cases of bronchial perforation, although its absence did not exclude it (17).

We have used fluoroscopy as well as roentgenograms taken both in inspiration and expiration to help diagnose bronchial obstruction to determine whether it is the cause of obstructive emphysema or atelectasis. Thereby, we have been able to demonstrate emphysema more frequently than appears to have been reported in the literature.

Our experience with roentgenographic signs of aspirated foreign bodies has impressed us with the value of films made in both phases of respiration. When incomplete obstruction of a bronchus is present, which allows air to enter the obstructed lung, the roentgenogram in inspiration may show no variation from the normal.

At the end of expiration, however, the air in the good lung has been expelled while that in the obstructed side has been trapped, clearly showing the increased air content of the stenosed side.

What is equally important, the mediastinal structures at the end of expiration

have shifted to the uninvolved side, being forced there, so to speak, by the affected emphysematous lung. This phenomenon is, of course, equally well, if not better, demonstrated during fluoroscopy. In obstructive emphysema the reverse of the Holzknecht-Jakobson sign is present in that shifting toward the stenosed side as seen at inspiration is not the active but is the passive phase of this process.

In atelectasis caused by obstruction of the larger bronchi, the heart and mediastinal structures are found shifted to the obstructed side in both phases, but more prominently so during inspiration. When smaller bronchi are involved, these roentgenographic signs will show variations.

Our thinking in respect to the need for bronchoscopy has been geared to the question which we have regarded as uppermost: Has perforation into a bronchus occurred?

Here it is worth while remembering that bronchial rupture usually occurs without appreciable rise in temperature or sedimentation rate. When these are elevated prior to rupture, they indicate a particularly active process. Here the bronchoscope simultaneously becomes a diagnostic as well as a therapeutic instrument. Bronchoscopy pinpoints the diagnosis and then can be useful in accelerating resolution.

If an area of thin mucosa with necrotic lymph node contents "shimmering through" can be demonstrated in a compressed bronchus, there need be no hesitancy to use strong suction to initiate the perforating process. We were impressed in a few instances in which this was attempted.

When fever and signs of activity were noted after known bronchial rupture, we looked for complications distal to the point of obstruction. Here it was hoped that by improving pulmonary ventilation and drainage, drowned lung, pneumonitis, and further bronchogenic spread might be controlled. In this connection, we used small suction tubes to aspirate material from the lesser bronchi.

Bronchoscopy should not be performed during acute upper respiratory infections or exanthemata. If, however, the fever is believed to be caused by the tuberculous process, bronchoscopy need not be a contraindication. Finally, the age of the child should not be a deterrent if small tubes are employed.

## BRONCHOSCOPIC TREATMENT

The assessment of the value of bronchoscopy in endobronchial childhood tuberculosis will be colored by the previous experiences of the bronchoscopist. His experiences treating aspirated foreign bodies, coupled with the success he has had in visualizing the sites of tuberculous bronchial perforations, will, of course, strongly influence his approach to the problem. Furthermore, the frequency with which he encounters caseous necrotic material and the character of this debris will have great bearing upon his recommendations.

Obviously, aside from its diagnostic value, bronchoscopy can only momentarily increase the patency of externally compressed bronchi. After perforation has occurred, we have frequently been able to aspirate or remove with forceps some rather large coherent caseous masses. We also believe that the small soft

necrotic pieces which we have often found could have been coughed up naturally.

Relative to the larger caseous masses which often have the consistency, size, and shape of aspirated vegetable foreign bodies, we believe that after prolonged sojourn these may also engender mechanical effects such as drowned lung and bronchiectasis. Jackson (11) stated that only about 2 to 4 per cent of aspirated foreign bodies are coughed up spontaneously. This would not bespeak a sanguine hope for spontaneous expulsion of all endogenous bronchial foreign bodies.

In reality, the crux of the problem is related to the stage of the caseation process that the lymph nodes already have undergone when perforation occurs.

It will be recalled that the mediastinal tuberculous node first necrotizes centrally. Next, the caseous material is broken up into large, coherent, elastic masses, some of which, as we have found, make an early exit through the bronchial wall. Later these become fragmented into smaller particles which ultimately coalesce into a cold abscess containing softened necrotic debris.

If perforation has been delayed until this stage, bronchoscopic treatment may not be necessary except to prevent spread to peripheral bronchi.

Endobronchial granulations may originate early from the endematous tissue changes near the periphery of the necrotizing node. When they appear in the larger bronchi, they may produce only mild symptoms. When they occur in the smaller branches, they may cause obstructive emphysema or atelectasis, and should be removed. No serious bleeding need be feared from the use of forceps or suction tube. We have not employed silver nitrate or other cauterizing agents.

Lastly, we have been guided by the hope that, if the obstructing masses and granulations are removed by bronchoscopy without delay, fewer segments and lobes will remain that later will fail to re-expand.

* * *

In summary then, bronchial involvement is an important and increasingly recognized factor in the pathogenesis of primary pulmonary tuberculosis in children. Bronchoscopy is the indispensable diagnostic method for visualization of the inflammatory or obstructive phenomena resulting from mediastinal lymph node involvement. Bronchoscopy in tuberculous children is not dangerous and is best performed with adequate anesthesia.

The indications for bronchoscopy are similar to those associated with foreign bodies aspirated into the tracheobronchial system. Frequently, the complications of bronchial involvement may require bronchoscopic treatment, which may also avert other undesirable pulmonary sequelae.

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## GENERAL DISCUSSION

Dr. Robinson: We have been seeing a lot of X-rays, and for that reason we thought it might be advisable to start this discussion of the papers by Drs. Lincoln, High and Nelson, and Laff by introducing Dr. R. Parker Allen, roentgenologist of the Denver Children's Hospital, Assistant Professor of Radiology at the University of Colorado School of Medicine, and pediatric roentgenologist at National Jewish Hospital.

Dr. Allen: Thank you very much, Dr. Robinson. It isn't very often that the bat boy gets up between innings, but that is about the way I feel here. I don't have any X-rays to show, so you can relax.

I would like to make a couple of comments about the things I have heard here this afternoon, and then tell you just a little bit about our experience at Children's Hospital here in Denver. I especially would like to agree with Dr. Lincoln and others, too, in emphasizing that the changes from day to day in the X-ray picture are not something to rely upon in the treatment or in the prognosis for the future, either day to day, or month to month. We have been fooled by that too many times here at the National Jewish Hospital, and I believe it can't be emphasized too much.

Also, Dr. High mentioned that in his cases he found that relief from obstructive emphysema correction was more marked than the relief seen in correction of obstructive atelectasis. That has certainly been our experience too and emphasizes the point that has already been mentioned several times, namely, the importance of taking expiration films.

I would like to interject here, from the standpoint of the radiologist, that it is not true that you cannot get good expiration films and good inspiration films on any child at any age.

Sitting on the sidelines and looking in upon this group of experts discussing tuberculosis, endobronchial and lymph node, I was thinking about the fellow who is outside and who sees these patients before they get to these big centers. How does he tell that this is tuberculosis?

Of course you all know the answer better than I; but from the standpoint of the radiologist alone, aside from the great importance of the tuberculin test and the history of contact, we hear it said so frequently that there is nothing specific about the picture of tuberculosis in the infant and young child's chest, and yet this afternoon you have heard over and over again the mention of hilar nodes, obstructive signs, and so on.

Thinking about that, I reviewed the films that were made at the Children's Hospital, which is a general medical hospital not restricted to chest diseases by any means. During the last ten years there have been 2,600 chest films made on sick children less than five years of age, that is, children sick with some type of pulmonary disease. Fifty of these children who did not have tuberculosis showed enlarged lymph nodes. That is about 2 per cent. I mean large lymph nodes in which you could actually see the margin of the node; not something that you imagine under some other infiltration.

During the same time, 125 cases of tuberculosis in children less than five years of age were seen. I am excluding miliary tuberculosis and just a calcified node or a calcified parenchymal lesion.

Of these 125 children, 100 had unilateral lymph node enlargement with or without parenchymal disease. Forty of the 100 had obstructive signs of one kind or another, either atelectasis or emphysema. Inspiration and expiration films were taken in almost all of these cases. So, there is a pattern which is of definite suggestive value.

I would like to ask if there is time for Dr. Lincoln to answer how many out of her 147 cases showed definite lymph node enlargement at their earliest examination. Thank you.

DR. ROBINSON: Thank you, Dr. Allen. Dr. Lincoln, will you answer that question first?

DR. LINCOLN: I don't have the figure here, but I know it was less than 5 per cent who did not show definite lymph node involvement in relation to the obstructive lesion.

DR. BELA SCHICK (Brooklyn, New York): I don't know whether I should take part in this discussion or not, but about forty-seven years ago I published a paper on the subject of antibiotic or antitubercular treatment. I described at that time only the clinical syndrome which was so characteristic that you could make the diagnosis, so to speak, without examination—at least you could expect there were nodes.

The symptoms were the peculiar kind of cough and expiratory wheeze beside the positive tuberculin test. I would not have talked about it, but I thought you would be interested in the prognosis of such children.

We had about thirty-four. When you have written a paper forty-seven years ago you forget half of it. Sometimes a man of my age should take out his old papers and read them again. Maybe he has also forgotten his paper.

I know only so far not exact data, but thirty-three or thirty-four children had the typical syndrome. At that time Schlossman published a paper on tuberculosis in the first year of life as being 100 per cent fatal. Then I was a little peeved about his remark, and we were a little more optimistic. There was, therefore, interest in the thirty-four children who did not die but who survived. Not all, but about one-third of them, survived.

The interesting thing was that these children recuperated entirely, in spite of this very serious picture of the child recuperating and recovering entirely without any X-ray symptoms. These atelectases disappeared, and all the symptoms disappeared.

DR. GIRDANY: As director of the X-ray department at a large children's hospital, I should like to add the following comments.

First, in regard to Dr. Allen's remarks: In our experience, at least, it is difficult to obtain satisfactory inspiration-expiration film studies of infants and children. You can take a film in inspiration, and you can take a film in expiration. Spurious changes are common in the absence of proper and equal positioning on both films. Fluoroscopic examination is the

more useful method of determining obstructive emphysema and changes in aeration in the lungs; this does require an experienced examiner and a child properly restrained or controlled.

Although there may be changes on roentgenograms of the chest that are highly suggestive of primary tuberculosis, the shadows are not specific. Rarely should they be used by themselves to establish the diagnosis of primary intrathoracic tuberculosis.

The attitude that we take is summarized in the reading, "There are shadows of increased density in a given area of the chest. If this child has a positive tuberculin test, and if these changes persist, then they are consistent with the diagnosis of primary intrathoracic tuberculosis."

I would like to suggest that this is a proper time to consider the role of the radiograph in the management of the patient with primary tuberculosis. Before such a distinguished audience, these remarks may be presumptuous; nonetheless, I cannot help but feel that the radiograph is given too much importance in the evaluation of the clinical condition of the infant or child with the disease.

Such terms, for example, as "infiltration" have been repeatedly used in discussion here. Save in those children more than six years of age described by Dr. Sifontes, and in other rare instances, infiltration is not a part of the disease in the lungs of children. "Infiltration" is a term that should properly be restricted to the disease in adults, in whom it refers to stringy shadows in the lungs and implies actual multiplication of tubercle bacilli with reaction in the lungs around them.

The roentgenographic changes in the lungs of the infant and child usually reflect either atelectasis resulting from endobronchial disease or the perifocal exudative reaction in the parenchyma associated with altered sensitivity to the tubercle bacilli. Only in disseminated miliary disease and in bronchogenic spread may the shadows reflect parenchymal reaction to multiplication of the bacteria.

It may be, Dr. Lincoln, that the absence of correlation in the size of the shadows in those instances in which they did not parallel the bronchoscopic changes can be explained on the basis that the large shadows reflected perifocal exudative reactions in the lungs themselves and were not related to compression or obstruction of the segmental bronchus.

One final comment: The use of the term "progressive primary tuberculosis" is one that, as a radiologist and pediatrician, I do not completely understand. If it is used to describe the waxing and waning shadows in the thorax, it is not being used properly. The fluctuation in size of shadows within the chest does not necessarily correlate with the clinical condition of the patient. To me, "progressive primary disease" indicates tuberculosis in a child who cannot handle the disease and shows unsatisfactory clinical progress. The use of this term to refer solely to the size of the shadows in the chest does none of us credit.

Thank you very much for your indulgence.

DR. NELSON: I happen to have heard by way of the grapevine that Dr. Janet Hardy has in her hip pocket a couple of slides—just by chance. I would like to make a motion, if it is in order, that she be given the opportunity to show them.

While I am on my feet I would hope that Dr. Terplan might be given a chance to say a word or two in regard to two of Dr. Girdany's remarks, one about tuberculous infiltrations and the other about what might and might not be progressive tuberculosis. I will leave out the word "primary."

In order to save time, I might also point out that, in contrast to the procedure employed by Dr. Laff, with which I am not taking issue, but simply for the sake of the record, none of our patients received general anesthesia; and practically none of them, local anesthesia, at least not in our own unit.

We feel quite comfortable, both from the standpoint of handling the child and psychologically, in doing without. I might add that I have been bronchoscoped myself, and it is much more terrifying to watch than to have.

DR. JANET HARDY (Baltimore, Maryland): I do have some slides, but I do not think they are pertinent. However, I would like to make a comment or two which might be pertinent.

There has been considerable discussion of the use of steroids in the treatment of tuberculosis. I know that a number of people here are going to go home and try cortisone in the treatment of endobronchial disease.

We have a very tiny experience in this field. It consists of a series of only four cases, but cases which have been very dramatic.

The first one was a baby who was almost moribund from the suffocating effect of tracheal compression due to large lymph nodes at the carina. This baby had also severe diffuse endobronchial tuberculosis with edema and ulcerations in the tracheal mucosa. The right main bronchus was about at the point of rupture of a tuberculous lymph node. This was several years ago and the baby was given ACTH. Because he was so ill, the ACTH was given intravenously. Within a matter of twenty-four hours it was possible to get the infant out of oxygen. Within a matter of two days the endobronchial lesions were receding; and in about five days the mucosa of the trachea and the main bronchi looked practically normal except right about the point of perforation. The baby was treated to the point where tuberculin hypersensitivity could no longer be demonstrated by skin testing. The ACTH was discontinued, and he promptly relapsed. Treatment with ACTH was again instituted before the relapse had progressed very far, and again the disease regressed. This time there was no recurrence when treatment was stopped. This infant was, of course, treated with streptomycin and PAS in the usual dosage for a prolonged period.

We have treated three other children with cortisone. The lesions were less severe. The first two of these had rather short courses of treatment, and no evidence of relapse of endobronchial lesions occurred when treatment was discontinued. The third one had a relapse the first time, and required a second course of treatment. These children were treated with isoniazid and streptomycin in the usual fashion.

The change in the bronchial mucous membranes following treatment with steroids in adequate dosage can only be described as dramatic; the disappearance of edema, inflammation, granulation tissue, and ulcerations, when present, seems to be remarkably complete in a very short space of time.

I would like to ask Dr. Lincoln a question, if I may. We have observed a series of people with bronchiectasis following primary tuberculosis; we have about fifty such children now. Some of these had their tuberculosis many years ago and actually are in their twenties now. We have half a dozen, Dr. Lincoln, who have severe saccular bronchiectasis in either a whole lung or in one lobe of a lung who have never had any symptoms. We have not submitted these patients to surgery because they have seemed to stay entirely well, and the surgeons have felt that the operation might be rather difficult. We do have a series of patients who have been submitted to surgery and who have done very well, but these have all been people who had symptoms of secondary infection.

I wonder if your indication for surgical treatment is secondary infection, or if your indications are more liberal than that.

DR. LINCOLN: I agree with Dr. Hardy that most of the patients with bronchiectasis following endobronchial disease do not have symptoms.

Our purpose in removing segments or lobes containing areas of bronchiectasis is partly experimental since we cannot know whether these patients are going to have trouble later on.

In general, our policy has been to advise resection of bronchiectatic areas which occur in the middle lobe, in the lingula, or in segment 6 of either of the lower lobes. These are the sites which most frequently give rise to symptoms in adult life, such as the right middle lobe syndrome.

This policy is based on our experience prior to specific therapy. In patients with extensive bronchiectasis it was our practice, as I am sure it was with Dr. Hardy, to have surgery done whenever we could; but inevitably there was a control group of the patients who refused surgery.

Some of these patients have continued, as in Dr. Hardy's group, to do very well. In fact, we had one boy who had a massive bronchiectasis since he was a baby and who has

been in prison for the past five years, and the prison doctor apparently didn't even know that he had bronchiectasis.

However, we have found that a certain number of these individuals, when they reach adult life, get into difficulty either with secondary infection or with hemorrhage. It is for this reason that we advise resection of extensive bronchiectasis or of bronchiectasis of any degree which occurs in areas which do not drain well.

DR. GORDON: As a thoracic surgeon, I should like to see the large residual primary foci removed and excisions made around the so-called drippings, so that there would not be any difficulty with organisms that won't respond to chemotherapy.

I should like to indicate just two points, one which Dr. Lincoln has already answered in part. I shall not hope to have the advantage of coming back forty-seven years later and talking about a paper that I wrote, as Dr. Schick has just nostalgically done. I did write a paper distinguishing between tuberculous and nontuberculous bronchiectasis.

Before taking that up, however, I should like to say that the persistent bronchial lesion, referred to by Dr. Lincoln, today is not unlike the persistent bronchial lesions that we had before the era of chemotherapy. They are an index of incomplete healing of a focus, parenchymal or lymphogenous.

In the years when we were doing thoracoplasties, pneumothorax, phrenic nerves, and so on, if there was a persistent bronchial lesion, it meant to me that the incompletely healed bronchial lesion indicated failure of healing of the tuberculous foci. Recent studies of resected material have borne this out quite well.

We have seen in these discussions here that the lymph node poorly responds to chemotherapy, that in excisions and in examinations teeming organisms or viable organisms of one type or another have been found in the lymph node and the pulmonary segment. Therefore, when you have greatly delayed healing of a bronchial lesion, it is an index of incomplete healing of the tuberculous foci.

Referring to bronchiectasis, the difference between the two patterns that you have just talked about is this: When the parenchyma of the lung is involved, as in tuberculosis, and you see bronchiectasis, it is not the same pattern as when you see ectasia, secondary to collapse of a lung due to lymph node enlargement, foreign body, or contiguous lymph node obstruction. These middle lobe syndromes which you see—the fact that a lung or lobe or segment becomes drowned or incarcerated and does not re-ventilate—then you see a picture of an inactive portion of respiratory tissue.

Normally the bronchial tree, in respiration, as you all know, elongates and widens in inspiration, which is a little different from other tubular systems, and in expiration it shortens and narrows. When the respiratory function is lost by obstruction, whether it be intrinsic or extrinsic, you see airlessness—to avoid the term "atelectasis." When the airless lobe becomes incarcerated and does not re-ventilate, then you see the pattern of arrest in respiratory movement as shortening and widening of the bronchial tree.

These children, with post-tuberculous atelectasis, may go on, as you have heard, for many years without any symptoms. They do not have the characteristic picture of bronchiectasis, which so often starts in early childhood with an exanthema. They have an infection at the outset and they continuously go on and cough and raise and perhaps spit up blood.

These children may not have any symptoms at all. At some later date, they have a lower respiratory illness; they present themselves to the internist, and then, after careful study, the ectasia is demonstrated in that part of the lobe that has never re-ventilated but which now only has had a superinfection.

That is an entirely different picture from the early infectious process in which we see the classical picture of bronchiectasis.

DR. USTVEDT: I would like to hear the voice of Dr. Middlebrook, who is an expert on the theory of therapeutic failures, as to what his views are. Why don't we get the usual response in these lesions? Is it a question of inadequate dosage, or what is it?

DR. MIDDLEBROOK: I thought there was such a phenomenon as you suggest—the phenomenon of failure of enlarged lymph nodes to get smaller—tuberculous lymph nodes to get smaller. The first thing that I think about are the observations of Dr. Schmidt—Dr. Schmidt's observations in monkeys—true, most of the experimental monkeys had not had their tuberculous disease very long before they were submitted to chemotherapy.

As far as the monkeys that have had their disease for some time are concerned, I think it would be very profitable to hear from Dr. Schmidt with regard to what happens to the mediastinal lymph nodes in the animals so treated with antimicrobial agents.

DR. SCHMIDT: I want you to forget about the things which I said yesterday about the naturally acquired disease—and turn your attention to our experience in induced pulmonary tuberculosis, in which we have been able to ascertain the status of the infection at the time treatment was initiated by sacrificing groups of animals which, radiologically at least, seemed to have the same extent of pulmonary and nodular involvement as those subjected to treatment. At the time treatment was initiated in this group of animals, there was already marked hypertrophy of the tracheobronchial lymph nodes and an extensive amount of caseation in certain of these nodes.

There are certain distinguishing features which accompany the different therapeutic regimens in so far as the progression or regression of disease in these nodes is concerned. Irrespective of the dose of streptomycin which is given, whether it be 5 mg. per kg. (which is definitely inadequate) or a dosage of 80 mg. per kg., which is the largest amount that one can give to a monkey without getting evidences of central nervous system intoxication, one always ends up terminally with very tremendous involvement of the tracheobronchial nodes.

The situation is not too much different with treatment with 5 mg. per kg. doses of isoniazid but, when one gives what we consider to be maximally effective doses, which are 80 mg. for the monkey and which correspond, in terms of effective drug, to 20 mg. per kg. in man, the distinguishing thing is the apparently normal structure of the tracheobronchial nodes.

My colleague, Dr. Jolly, who is the pathologist in our group, could probably add considerably more to the discussion of the pathologic pictures in these nodes.

DR. PAUL N. JOLLY (Cincinnati, Ohio): To go into great detail about these pathologic changes, I think, would be superfluous at the moment. The basic variations have been stated percentage-wise.

About 90 per cent of the adequately treated monkeys who received isoniazid showed no residual evidence of the tuberculosis. We could not recognize anything as a tuberculous infection. Occasionally scars will be present, but no tubercles, no giant cells, and the like.

The streptomycin animals, on the other hand, will almost universally show evidence of infection which can be recognized as definitely tuberculous in origin.

DR. GRZYBOWSKI: I just want to touch on one or two problems that were mentioned by previous speakers. What I am going to say relates to the experience of Dr. Bentley and myself in the follow-up study at High Wood Hospital. We have followed up 169 children with segmental and lobar lesions.

The first point I would like to touch upon is the radiological course of these lesions. When you observe these lesions for a long period of time you see the gradual contraction of the shadows cast by the affected segments going on for as long as eighteen months or two years, and then in most situations the shadow is no longer visible in ordinary projections.

To find out exactly what happens to these lesions, we have chosen the anterior segment of the left upper and the anterior segment of the right upper lobes, which, as Dr. Lincoln has pointed out, are so frequently involved. I would like to show a slide which may illustrate the roentgenographic course better than I can express it in words.

Figure 1 is an X-ray of a child about two years of age who in 1942 had a segmental shadow involving the anterior segment of the left upper lobe. One could see subsequent gradual contraction. The second picture shows quite marked contraction in two years and, finally, the picture taken six years later shows the common end result of segmental lesion.

FIG. 1 (Top). Roentgenogram of a two-year-old child with segmental shadow involving the anterior segment of the left upper lobe.

FIG. 2 (Center). Roentgenogram obtained two years later, showing marked contraction.

FIG. 3 (Bottom). Roentgenogram obtained six years after figure 1, showing common end result of segmental lesion.

Of thirty-two patients with lesions of the anterior segment either on the left or on the right, we have seen this end result in thirty.

Figure 4 is reproduced from *Tuberculosis in Childhood and Adolescence* published by NAPT, London, 1954. The end result of a contracted vacuolated segment is well shown in the planigram. This is a segmental lesion of a child who had what we could call a consolidation of the anterior segment of the right upper lobe some four and a half years previously.

Second, I would like to say a word about the prognosis in this condition. Of 169 children with these large opacities, we found that the incidence of tuberculous complications was

FIG. 4. Planigram showing a contracted vacuolated segment.

*(Reproduced from Tuberculosis in Childhood and Adolescence by F. J. Bentley, S. Grzbowski, and B. Benjamin, by permission of the authors and of the publishers, the National Association for the Prevention of Tuberculosis, London, England)*

not appreciably higher than in the groups showing only hilar adenitis or primary complex. These large lesions do not seem to have any marked prognostic significance.

What about bronchiectasis? We found that you could almost pick up the cases in which symptomatic bronchiectasis will develop. In the last case which I showed, undoubtedly there were dilations of the bronchi, but the child was symptom free.

Only 10 of our 169 children developed bronchiectasis as a clinical condition, with cough and sputum. All of those 10 children initially had lesions involving large areas at the base, for instance, right lower lobe and right middle lobe or left lower lobe.

In conclusion, I would like to say that these two facts, the very slow roentgenographic progression of these lesions and the good prognosis in those children, should be taken into account in the management of this condition. I refer particularly to the surgical treatment, which, apart from the exceptional cases and apart from cases treated surgically for very interesting experimental or pathologic investigations, has not much place in the treatment of this condition.

DR. TERPLAN: I would like to make a few brief historic comments: Dr. Schick, in 1910, pointed to the clinical symptoms brought about by the constriction of the bronchus from the enlarged tuberculous lymph nodes, in particular to respiratory wheezing. Dr. Fleischner, also in Vienna, was among the first to correlate extensive lobar atelectasis with bronchial constriction, by bronchoscopy. Ghon and Epstein were the first to shed light on the term "epituberculosis," which had been introduced as a clinical entity by Eliasberg and Neuland. They proved that the anatomic change responsible for the X-ray shadow of this "epituberculosis" was obturation atelectasis of an entire lobe, secondary to tuberculosis of the major bronchus leading to the lobe.

Since then, we have learned that tuberculous lesions might or might not develop in the atelectatic lobes following compression of the bronchus or occlusion of its lumen from endobronchial tuberculosis.

In 1936, Roessle published his well-known paper, dedicated to the seventieth birthday of Dr. Ghon, on the anatomic analysis of eight cases in which the clinical diagnosis of "epituberculosis" had been made, stressing, in particular, the compression of a large bronchus by one or more caseated lymph nodes. On this concept, namely, the bronchial obstruction in primary pulmonary tuberculosis in children in its relation to "epituberculosis," many other studies were based, including material published from our own department in Buffalo.

I wanted to make this brief comment because the original observation from Dr. Rich's laboratory by Oppenheimer was of a different nature, stressing "resolving tuberculous pneumonia" as the cause of epituberculosis. Only later, in some reports included in his book, taken from incidental post-mortem findings in adults but including also an observation in a child, obstruction of the bronchial lumen following perforation of the tuberculous lymph node has been reported by Rich.

Also a few words about the findings of Dr. Phillip Schwartz, so frequently cited of late: In Dr. Ghon's original monograph on the primary pulmonary focus in 17.75 per cent (in 30 cases among 169), rupture of a caseated lymph node into the bronchus was observed. This lymph node was usually regional to the primary tuberculous focus. This was observed in particular in the more rapidly progressing primary lesions leading to combined intrabronchial and hematogenous dissemination.

In the material from Buffalo, included in the presentation given here yesterday, four such observations were shown.

So, all this is well known all over the world and had been observed more than fifty years ago in France as well as in Austria.

Contrary to the opinion of Schwartz, in the pathogenesis of chronic pulmonary tuberculosis in the adult, as observed in our sanatoriums here, and as we have studied it in Buffalo and in Prague, this phenomenon, namely the rupture of a caseated lymph node into a bronchus, has very little, if any, significance. Obviously, Dr. Schwartz's experience in Turkey was quite different. We can demonstrate, occasionally, scars in the wall of the major bron-

chus, obviously caused through perforating lymph nodes, largely as incidental findings without any significant tuberculous involvement of the lungs. Also, nontuberculous lesions, in particular those caused by anthracosis with marked liquefaction, can lead to localized perforation into the bronchus, leaving extensive anthracotic discoloration in the scarred mucosa behind.

I have been asked, also, to say a few words about whether or not "infiltration" is a justifiable diagnostic clinical term. That infiltration of the lung tissue in progression of a recent tuberculous infection can occur is obvious. I have demonstrated several pictures showing such a progression via bronchi in the lung, producing gradually enlarging shadows on the X-ray film. I take it that the objection of Dr. Girdany to the term "infiltration" is that in the particular condition under discussion we are usually dealing with atelectasis and not with a spreading infiltrating process.

If Dr. Nelson wants me to say what I consider "progressive tuberculosis," I have to say that progressive primary tuberculosis is a primary tuberculosis which is steadily and continuously progressing.

Most of what we discussed today is really not progressive tuberculosis, even if the lymph node changes are still active in a histologic and biologic sense. However, the interesting thing is—and again pardon me for reminding you of some of our own experiences, some fifteen years ago—that children in the acute phase of obstruction might appear quite sick, in addition to the alarming massive X-ray shadow caused by atelectasis of the lobe. However, once the obstructing material, consisting of caseated lymph node tissue debris, is removed, they soon return to perfect health, with the pulmonary shadow gradually clearing. In several such instances, the diagnosis of aspiration of a foreign body, food particles, et cetera was made. In all of them, tubercle bacilli could be demonstrated in the material removed by our bronchoscopist. We have followed the course of these children. They all remain well. I had two similar experiences with young adults in whom most probably the primary infection was a relatively recent one, causing localized perforation of the major bronchus with massive atelectasis. Following the removal of the obstructive material, the patients recovered quickly and have remained well ever since.

To return once more to the definition of "progressive primary tuberculosis," it includes the classic recent primary complex, causing lymphohematogenous dissemination, more or less rapidly or in a somewhat protracted fashion, but still continuously progressing. Or, it is a rapidly spreading lesion with cavitation of the primary focus and massive infiltration by direct extension and through the bronchi, involving the entire lung or both lungs. Destruction of lung tissue from cavitation might preclude extensive lymphohematogenous miliary spread. I showed you a few cases of this type yesterday.

One interesting additional observation I would like to mention here. Whether or not it has any significance, I do not know. In cases treated with isoniazid in the Lockport Tuberculosis Sanatorium, I have been impressed, during the last two years, with the fact that some by no means very large tuberculous ulcerations in the intestine have healed with considerable fibrous constriction of the lumen, causing stenosis.

I was happy to hear Dr. Lincoln state that in one of her cases of bronchiectasia, no treatment was given and that the result was not different from that in the other group of cases under specific treatment. The only question I would like to pose for discussion is whether or not rapid fibrous regression of the enlarged caseated lymph nodes and their capsule, particularly including the bronchopulmonary lymph nodes firmly attached to the wall of the bronchus, might be to some extent responsible for stenosis or bronchiectasia. It would be interesting to see whether the incidence of bronchiectasia has changed more recently as compared with that of the pre-antimicrobial era.

DR. CANETTI: I just want to say a very brief word on the bacteriologic condition that exists in those perforated lymph nodes. I think this may have a little bearing on the question which Dr. Ustvedt asked about the reason for the nonhealing of these lesions by chemotherapy.

I would say that although, of course, cases exist in which these perforated lymph nodes are teeming with bacilli, in my experience they usually do not. It is surprising to see that such perforated lymph nodes are usually rather poor in bacterial content, which is a characteristic of theirs which distinguishes them in the most important manner from, for instance, a lung cavity.

A lung cavity teeming with bacilli, a lymph node cavity, so to speak (because it is not exactly a cavity), is absolutely nothing. That is why this condition of perforation of lymph nodes in top bronchi is, bacteriologically speaking, not a very dangerous thing. That is why one usually does not have them seeping into the lungs under such conditions. That is a rather rare occurrence.

When a sudden perforation occurs, the problem is rather a mechanical one, as all these distinguished papers we have heard have stressed.

So, the same reason which makes these lymph nodes a very poor bacterial thing, if I may speak so, shows at the same time why they do respond so poorly to chemotherapy. It is just not a bacterial question but a question of residual necrosis which has to heal by itself, and eventually it does heal but, of course, rather slowly.

Dr. Middlebrook: "Cavité." It is not the same in English. That is what we were getting at the other day. I want to be sure that what Dr. Rist was talking about was a "caverne," in French, in the lung, which opened into the bronchus, and in general in English we speak of "cavities" as you speak of "cavernes." We don't usually use the word "cavity" for an abscess, regardless of the nature of its wall or the nature of its contents.

Dr. Canetti: In this case it is a cavity.

Dr. Gladys Boyd (Toronto, Ontario): Since Dr. Lincoln was kind enough to refer to the fact that I have thought you did get better results with isoniazid, I would like to say why I think so. Perhaps it is because I am unorthodox enough to use only isoniazid in these cases, and no other antituberculous drug, but I don't think that is the answer.

We have been doing bronchoscopic studies of tuberculous bronchitis since 1929. The first pulmonary resection for this disease was done in that year. The investigation has been carried out by two otolaryngologists, successive heads of their department at the Sick Children's Hospital. The procedure was carried out under general anesthesia to allow an unhurried investigation. More than 300 children have been studied.

The project could be divided into three periods: (*1*) 1929 to 1946, when streptomycin became available; (*2*) 1946 to 1952, when streptomycin with PAS or Promizole® was used; and (*3*) 1952 on, when isoniazid alone was given as a drug. In all three groups, bronchoscopic studies plus removal of any protruding granulation have been carried out. In the first period some patients did recover after lengthy illness and repeated removal of the obstruction; more needed subsequent resection. Twenty-nine resections were done in the second period in those cases where either fibrotic lesions or granulations persisted. The operative results were good. In only one resected specimen was there a total lack of persistent tuberculous disease, as proved either by the pathologic picture or continued evidence of active infection as indicated either by culture or animal inoculations of the specimen. In endobronchial studies of these cases, after isoniazid became available, it soon became apparent that, once the granulations were removed, even if excessive, they most frequently did not recur or at most only needed to be snipped off once or twice subsequently. In many patients, the disease disappeared. Five resections were done. In none of these was there bacteriologic or anatomic evidence of persistent disease. Two patients had airless shrunken lobes which were considered harmless.

I might mention one lung that was removed in March, 1955, because I think it is of interest. This particular child had been treated for tuberculosis with streptomycin alone six years before in another sanatorium. He had a lesion in his left upper lobe when he was discharged, but it was considered to be calcifying, and they did nothing about it. He came to our hospital last winter for the treatment of his deafness. He has positive sputum and positive urine, so we put him on isoniazid and streptomycin. The lobe was resected last

March. It is of interest that, although we found acid-fast organisms in the specimen that stained readily, to date (using both mice and guinea pigs) we have been unable to grow anything, and all the cultures have been completely negative.

* * *

DR. ROBINSON: Thank you, Dr. Boyd.

Now we have the privilege of hearing from Dr. Schick, eminent pediatrician and investigator.

## VIEWS IN PERSPECTIVE

BELA SCHICK[1]

I find myself in a difficult situation, similar to that of Dr. Dubos. My talk was originally scheduled at the beginning of the meeting after Dr. Dubos' introductory remarks. Later, I discovered that the title of my talk was changed and that it was placed at the end of the meeting. This made my prepared remarks irrelevant. The new title is "Views in Perspective"—a title calling forth a certain amount of mystery. Happily, it permits me to talk about whatever I like; and, as was the case with Dr. Dubos, there will be no discussion.

My qualification to talk here is based partly upon many years of work at Sea View Hospital and partly upon the fact that I was one of the first to introduce BCG vaccination in New York City. With Dr. William H. Park and Dr. Kereszturi, I took part in the International Congress in Oslo. The vaccination interested me because it gave me the possibility to study an artificially produced first infection with tubercle bacilli in human beings. We found that the intracutaneous injection was preferable to the subcutaneous injection. The appearance of the positive tuberculin reaction could also be observed.

Good follow-up work is exceedingly difficult to do in New York City. The inhabitants frequently move and often cannot be found. Statistics are, therefore, difficult to evaluate. In France, Calmette also had great difficulty in obtaining reliable statistics. Since in our study the difference between the protection in vaccinated and control cases was very small, it was difficult to arrive at a definite conclusion; but no harmful effect was observed. In the early days, Petroff repeatedly pointed to the danger that the attenuated BCG bacillus might become virulent. The fight of Petroff against Calmette has been, I think, definitely settled in favor of Calmette.

The tragedy of Luebeck, where so many children died, was found upon investigation to have been caused by the mistake of using virulent human tubercle bacilli. It is easily understood that this unfortunate affair retarded the general use of BCG vaccination. The tragedy, however, gave a rare opportunity to study the course of tuberculous infection by intestinal invasion. A classical study of clinical pathology was published by Kleinschmidt.

I have been very much interested in this meeting. As a clinician I enjoyed listening to the excellent reports of so many outstanding representatives of laboratories conducting studies in tuberculosis. I congratulate the National Jewish Hospital at Denver for having arranged and organized such an excellent meeting.

One of the questions which I was eager to have answered was discussed yesterday by Dr. Debré. Everybody agrees that miliary tuberculosis and tuberculous meningitis must be treated. The question is: Should the first infection type of tuberculosis be actively treated with antimicrobials which are so very effective?

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Debré is in favor of treating infants and children up to three years of age. This age group is known to have the greatest tendency of generalization and is, therefore, in danger of developing miliary tuberculosis and tuberculous meningitis.

I would like to have heard something more about the disappearance of scrofula. Whereas I saw many such cases in my Vienna days, I have hardly seen a case since my departure. We defined scrofula as the second or generalization stage of tuberculosis plus X. I still do not know what this X is. We considered bovine tuberculosis and the importance of vitamins, secondary infections in unsanitary environment; but all these factors are unsatisfactory explanations.

Also of interest is the rare occurrence of phlyctenae in the conjectiva which we thought was a pathognomonic sign of active tuberculosis and of diagnostic value equal to a positive tuberculin test.

After deliberating what I should discuss, I decided it would be worth while to talk about a remark made by Dr. Dubos. He said that many phases of tuberculosis and the status of immunity were judged according to the behavior of the tuberculin test. Dr. Dubos questioned the use of the test as a measure of immunity and finally challenged the conception that allergy means immunity. There exists such a confusion about the relation of allergy to immunity that I, as the collaborator of Pirquet, feel it to be my duty to clarify the situation. Let me start with the history of the tuberculin test.

As you know, in 1890 Robert Koch published his fundamental observation that a person with active tuberculosis reacts to a subcutaneous injection of 0.01 ml. of tuberculin with fever, whereas an apparently healthy individual shows no rise in temperature. Koch further maintained that the normal individual would develop fever if larger doses of tuberculin were injected. Koch did not know that the fact that the temperature of a normal individual entirely free from tuberculosis will not rise after an injection of larger doses of tuberculin was due, as proved by autopsy, to the existence of an inactive, arrested tuberculous focus. Naegeli and other pathologists found such foci in nearly 100 per cent of adults. Koch tried to explain the difference in sensitiveness to tuberculin in the following way: A patient with active tuberculosis produces, during the course of his disease, a certain amount of tuberculin, which is added to the amount of injected tuberculin, which by itself would not produce a rise in temperature. By the injection of 0.01 ml. of tuberculin, the threshold for a temperature reaction is reached. The theory of Koch was, therefore, called the "addition theory."

My former chief, Escherich, was very much impressed with the diagnostic value of the Koch reaction and studied it in children. He made the interesting observation that some of the children who had a fever reaction to tuberculin developed a local inflammatory reaction at the site of the injection, which he called "Stich reaction." This reaction was also observed by Epstein. However, neither Escherich nor Epstein paid particular attention to this phenomenon, which was really a specific reaction to tuberculin.

Koch then started to study the therapeutic use of tuberculin. At that time one could hardly open a medical journal without finding articles about tuber-

culin therapy. This enthusiasm lasted for several years, but finally declined. Possibly it was dropped too early, at least before all interesting details were clarified.

I come now to the history of the cutaneous test of Pirquet. Everybody who wants to understand allergy must go back to the original observation of Pirquet which led to its discovery.

The most basic phenomenon in this connection is the existence of an incubation time in serum sickness. These observations required no large laboratories and almost no equipment. What was needed was a genius, and this genius was Pirquet.

Incubation time means the time which elapses between the moment of invasion of an infectious agent and the appearance of symptoms of the disease. In this connection, we have to differentiate between exotoxic and endotoxic diseases. An exotoxic disease like diphtheria has an incubation time of relatively short and variable duration. This time is required to produce that amount of toxin which will cause the threshold for clinical symptoms to be reached. This time varies somewhat, depending upon the rapidity of toxin production and the potency of the toxin.

In an endotoxic disease no specific toxin is excreted. The toxic substance is inside the bacterium. The bacterium multiplies at first without interference. Something has to be produced which will enable the organism to get rid of the intruder. Bactericidal substances are produced within eight to twelve days which kill the invading germ and set the endotoxin free. Now the disease starts. This is the mechanism aimed at eliminating the invader. We pay with the disease for our cure. The incubation time is needed for the mobilization of the antibodies.

Such an incubation time of eight to twelve days exists also in serum sickness. We cannot use the same explanation for the two types of disease just discussed. Serum as such is not toxic. There is no exotoxin and no endotoxin. Serum does not multiply. It is a dead substance. Something must be produced which changes the serum to a toxic substance. Serum is a foreign protein. We cannot tolerate foreign protein in our system; it must be destroyed. During this destruction toxic substances appear which produce the symptoms of serum sickness. Specific antibodies must be formed which break down the foreign protein in a way similar to the destruction of protein by digesting ferments in the intestinal tract. Serum itself, as I mentioned, is not toxic. Even after an injection of 100 to 200 cc., no immediate symptoms appear. During digestion of protein, toxic intermediary substances (like polypeptides, et cetera) appear. Finally the protein is broken down to amino acids which are again not toxic. Whereas in the intestinal tract the intermediary products are detoxified by the intestinal wall and the liver, these intermediary products are not detoxified if the foreign protein is injected subcutaneously because the digestion by antibodies occurs parenterally. Thus, by interaction of antigen and antibodies the toxic substance creates the symptoms of serum sickness.

That antibodies are mobilized against foreign protein can be proved by demonstrating precipitins. Purposely we did not identify the precipitin as the antibody which creates the clinical symptoms.

Pirquet observed that a child who reacted after eight days to the first injection of horse serum reacted immediately to a reinjection three weeks after the first injection. The incubation time was eliminated. Pirquet explained the change by the fact that antibodies were still present from the first injection. The organism had not to wait for mobilization of antibodies. The persisting antibodies could, therefore, immediately react with the reinjected serum proteins.

Antibodies disappear after three to four months; but the cells of the organism still remember how to mobilize specific antibodies. They now appear quicker than after the first injection, four to six days after the reinjection. Now, the symptoms of serum sickness appear after a shorter incubation time. The organism shows an accelerated reaction. This peculiar change of the incubation time is of utmost importance. The serum has not changed. It must be the organism which has changed; its reactivity has changed. That was the reason for Pirquet to coin a new term for this change. This term was "allergy."

In the case of serum sickness allergy exists without immunity. Unfortunately, frequently the immediate and accelerated reactions are intensified and can even become life-endangering reactions in the form of the so-called anaphylactic shock. We see, therefore, that allergy can exist without immunity. So far the statement of Dubos is correct. In the case of serum sickness, allergy does not lead to immunity.

However, in bacterial diseases and, in particular, in endotoxic diseases immunity is based upon allergy. The invading germ can multiply undisturbed as long as the antibodies are not present. When allergy is established and the antibodies are immediately available, the latter can go into action immediately and, in case of loss of circulating antibodies, the more rapid reappearance of antibodies enables the organism to kill off the invading germ; thus, its multiplication is prevented. Only a small amount of endotoxin will be liberated which produces no clinical symptoms. If, in consequence of allergy, more rapid production of antibodies ensues, the invading germ has much less time for multiplication. A much smaller amount of endotoxin will have accumulated and, therefore, a much smaller amount of endotoxin will be set free. The symptoms of disease will be much milder; and the shorter the incubation time, the milder the disease. We realize that in this example immunity exists. This immunity is based upon allergy. The fact that the incubation time is missing or shortened has diagnostic value proving that the organism was previously in contact with the same microorganism. We understand why a positive tuberculin reaction reveals the existence of a tuberculous focus. Other experimental data give evidence that the immediate and accelerated reactivity may guarantee a certain immunity against a new invasion of tubercle bacilli. It must be admitted that to judge the intensity of immunity is very difficult. We know that the sensitiveness to tuberculin may be very intensive in case of renewed massive invasion of the tubercle bacillus or in case of a renewed activity in tuberculous foci. A longer quiescence in tuberculous foci will lessen the intensity of the tuberculin reaction.

Pirquet applied his discovery to many other infectious diseases, particularly to smallpox vaccination. Furthermore, he extended his study to the so-called idiosyncrasy to foodstuffs and to the symptoms of hay fever, asthma, and eczema.

For a limited time he was interested in the diphtheria toxin. However, negative findings in the study of this toxin caused him to drop this part of his investigations. This gave me the opportunity to take up the study of the diphtheria toxin from a different point of view.

I wish to emphasize that the forms of allergy already mentioned (hay fever, urticaria, eczema, et cetera) and sensitiveness to different foodstuffs and protein-substances also follow the line of serum sickness. This is a form of allergy without immunity and the treatment consists partly in avoiding the offending substances or in desensitization. This form of allergy is certainly of no benefit to human beings. On the contrary, it is an undesirable allergy; I proposed to call it a pathological form of allergy. We derive the greatest benefit from the "physiologic" form of allergy, instituted by nature for the fight against infectious diseases. That a similar mechanism leads, in the fight against foreign protein, to unpleasant and even dangerous symptoms does not lower our admiration for the beneficial effect of anti-infectious allergy which is our great savior from destruction by pathogenic bacteria. Nature could not expect that we physicians would inject foreign protein and drugs for therapy.

It is of interest that Pirquet at first did not like to accept the tuberculin reaction of Koch as an allergic reaction, whereas I tried to point out many important facts which were in favor of it. With Pirquet's consent I went to Graz to restudy the charts of children tested by means of the original method of Koch. Here I was able to re-establish the correctness of Escherich's statement concerning the Stich reaction. I published that the Stich reaction is a specific reaction proving the existence of active tuberculosis. Pirquet still hesitated to accept the Stich reaction as an allergic phenomenon. We tried then to inject tuberculin subcutaneously. Not knowing the correct dosage, we frequently got intense reactions. Later Pirquet used for his study of smallpox vaccination a specially constructed borer so as to produce an equal-sized scratch effect. This gave him the idea to use the borer for tuberculin testing as well.

At first diluted tuberculin was used but, as the reactions were rather weak, he increased the concentration and finally found that concentrated Old Tuberculin gave the most satisfactory results. The amount of tuberculin absorbed through the small test lesion is remarkably small. As we later found, it amounts to 0.1 ml. of a dilution of tuberculin 1:10,000. In cases of a negative Pirquet test, the intracutaneous application permits an increase in the dose of tuberculin, and in this way a positive reaction can be obtained in persons who are less sensitive to tuberculin. The intracutaneous method of testing was developed by Mantoux. This test became so popular that the name of Pirquet is sometimes forgotten and even unknown to medical students. There exist other modifications of the scratch test of Pirquet, among them the Moro test which is sometimes used. This test consists of the use of a salve containing tuberculin which is rubbed into the skin. A very practical and widely used modification is the Vollmer test, a patch test. Small squares of filterpaper are soaked in concentrated Old Tuberculin. These squares are then dried and pasted on adhesive plaster. The plaster is simply put on the skin. The reaction is read after forty-eight hours and con-

sists of a little redness and infiltration at the site of the filter paper containing tuberculin. The results are very satisfactory. The reaction corresponds to the amount of tuberculin in the Mantoux test in the dilution of 1:10,000. The Vollmer test has the advantage that the child does not have to be injected with a needle.

As already mentioned, injections of tuberculin subcutaneously and intracutaneously proved that tuberculin is absolutely not toxic for an individual entirely free from tuberculosis. Even an injection of 1 gm. of Old Tuberculin will be tolerated without a reaction. A positive reaction in an infant or child up to three years of age proves the existence of active disease. In older children a positive test indicates that the child has been infected with tubercle bacilli, but the infection may be quiescent or healing. In the age group of more than seven years the negative reaction becomes more important and significant, permitting the conclusion that tuberculosis is not present, a decision most helpful for a correct diagnosis.

Tuberculin testing stimulated the study of first infection-type tuberculosis, its pathogenesis, and pathology. The classical study of the primary focus by Kuss and Albrecht threw light upon the inception of the disease. The unique investigation of Ghon constituted the climax of the study of first infection-type tuberculosis. Ghon found that more than 90 per cent of this type of case showed the location of the primary focus in the lung. The primary focus was usually very small, frequently not larger than a pea, situated subpleurally. It must be realized that such a small focus cannot be detected either by percussion or by auscultation, or by X-ray examination. It is a pneumonia produced by the tubercle bacillus. The detection of such a focus is possible only by the tuberculin test.

The chemical constitution of tuberculin is still not known. Protein, polysaccharide, lipid, and waxy substances are present. Dr. Dubos and Dr. Middlebrook could have told you much more than I about it.

Some difficulties present themselves in including the tuberculin test in the group of allergic reactions. Tuberculin never produces hives; the tuberculin reaction is a somewhat slower reaction and, examined histologically, is inflammatory. It is difficult to find substances which can be identified as antibodies. An excellent proof for the allergic nature of such substances is the passive transfer (Prausnitz-Küstner experiment). Some positive results proving the existence of antibodies could be found with tissue culture material and with leukocytes. Only lately, I found an article in the *Annals of Allergy* (August issue of 1955) where the existence of passive transfer was described:

> Tuberculin contains two biologically active components. One is a polysaccharide which is actively antigenic. It appears to be a fraction of tuberculin which is absorbed upon the surface of erythrocytes and becomes the reagent for the demonstration of circulating antibodies in many cases of tuberculosis.

The experts of our symposium, Dr. Dubos and Dr. Middlebrook, can give us a profound discussion about the problem.

This fraction, however, does not evoke the delayed type of response so characteristic of the classical tuberculin reaction. This latter reaction is produced by another component of tuberculin which is a protein of low molecular weight known to us as PPD (purified protein derivative). Being thus marked as the component against which the allergic reaction of the tuberculous patients is directed, it has been the despair of the immunologists, because of its poor ability to evoke an antibody response which would permit its demonstration by any of the classical methods *in vitro* and *in vivo*.

The reaction using PPD can be demonstrated in tuberculous patients. It has been shown that these antibodies are in all likelihood directed against the tuberculin proteins. The protein substance must always be present, even if only in very small amounts.

Cole and Favour, working with tuberculin-positive guinea pigs, report that they have succeeded in obtaining passive transfer of the delayed type of reactivity against PPD with a subfraction of alpha globulin for the isolation of which they elaborate a new method. This fraction contains also the reactivity with Boyden's reagent. By contrast, the antibody to the polysaccharides is found in the gamma globulin fraction of plasma. It is important to note that the gamma globulin has an inhibitory effect upon the reactivity of the alpha fraction which carries the agent of delayed type reactivity. This may explain several puzzling features of previous investigations.

Finally, I wish to make a short remark about a clinical diagnostic problem. Everybody knows that the presence of calcified foci in the lung field as seen in X-ray films was interpreted as definite proof of a tuberculous process. We realize now that we have been wrong. Such calcified foci in the lung develop also in histoplasmosis, a fungal disease occurring in certain regions of the United States. Sometimes both histoplasmosis and tuberculosis are present in the same patient. Histoplasmosis was described for the first time by Christian. A skin test exists for histoplasmosis. If the tuberculin test is negative, a positive histoplasmosis test makes the differential diagnosis easy. If both reactions are positive the situation is more difficult.

I feel that we can be encouraged by the progress which has been made in the treatment of tuberculosis. This encourages us to look for further progress. It will still take a great deal of time before we shall see Traviata and Mimi only on the operatic stage and never encounter such persons amongst the people inhabiting the world.

## Summary Session

Saturday Morning, November 12, 1955

WALDO E. NELSON, *Honorary Chairman*

ROBERT H. ALWAY, *Moderator*

### PROCEEDINGS OF THE SUMMARY SESSION

The final session of the Symposium on Tuberculosis in Infancy and Childhood, held at the National Jewish Hospital at Denver, on Saturday morning, November 12, 1955, convened at 9:15 a.m., Dr. Waldo E. Nelson presiding.

CHAIRMAN WALDO E. NELSON (Philadelphia, Pa.): For reasons unknown to myself I have been given the privilege and, without any question, the honor of opening this last session.

It certainly is not my function to try to recapitulate the program but, in mulling over what has gone on, one thought comes out and I express it only in the hope that it may perchance stimulate some additional comments this morning.

It would be awfully difficult to say what has been most important. Certainly at this stage I would not attempt it. As a clinician, I share with Dr. Schick the feeling that those of us who think of ourselves as being primarily bedside doctors had the opportunity to listen to what some people are doing in taking care of sick human beings. Again, I don't want to single out individuals, but I think the paper by Dr. Sifontes is one I would like to have permanently so that I can share his experiences. It was most impressive.

It seems reasonably clear that the problem that we are confronted with is in the field of prophylaxis. Not to recapitulate how we are going to define prophylaxis, I think we all agree that we have to approach it from two standpoints. One is actual prevention of infection; and the other is prevention of what might be called the complications of the established infection.

But there is inherent in the discussion that has gone on a problem which seems of some moment, the kind that might perchance leave us not with fear but with serious concern. The problem is: How best can we handle a situation in which infection, incidence-wise, has in our lifetime come under a degree of control that is encouraging. If we were merely to accept the philosophy that the best kind of prophylaxis is prevention of infection, what do we leave as an inheritance for the generations to come? If it is advisable and desirable to leave in the circulating fluids and the cells of people some type of specific immunity, then we will have to define more specifically what is adequate immunity and, in particular, we must find ways of measuring it.

I have been told that I am sometimes too concerned about terminology; but I was rather pleased when the thought was expressed that perhaps we shouldn't speak of tuberculous allergy. I don't know what particular term one should use. It might be better simply to speak of tuberculin sensitivity or, if one wants to emphasize the point, of tuberculin hypersensitivity. But we must find practical ways of getting at the problem of the mechanism of immunity.

What will constitute, or what actually are the factors involved in, immunity and what specific measures are required to induce it?

Now, I say this in part because of a reference that Dr. Dubos made in his opening address—a reference to something that he said he didn't believe—and that had to do with the work of Dr. Palmer and Mrs. Ferebee. I am stating this with the hope that our moderator this morning will insist that they get up and be given the opportunity at least to put on the record what they have observed up to the present, because it seems to me that, whether it is right or whether it is wrong, it ought to be a matter of record at this Symposium.

Now, if you will forgive me for taking advantage of being up on the platform, may I now present to you the person who will be responsible for the morning's Summary Session. It is a privilege and an honor to present Dr. Robert Alway.

DR. ROBERT H. ALWAY (Denver, Colorado): I think Dr. Nelson has so far begun to play an important role as the honorary chairman. When I asked him to be the honorary chairman this morning, it wasn't picturing him in the usual role assumed by such, recalling him as a man given to great silences. Because of his long interest in tuberculosis, his membership on the American Academy of Pediatrics Committee on Tuberculosis, because of his authority—and I mean authority—as witnessed by his textbook chapter on tuberculosis, I thought he could serve a very useful function here this morning.

Maybe too many times by now comments have been made relative to the evolution of this—I think, very successful—Symposium. I believe you would be interested to know that, when we started out, it was going to be an international symposium. This is not on the title page, I notice now, but it is to a certain extent international because of the kindness of so many of you in traveling so far to come here. Nevertheless, there are some international aspects which seem not to have received sufficient emphasis thus far. They might be considered in some of our discussions this morning.

Dr. Middlebrook received a regret from the President of the Japanese Tuberculosis Association in which he pointed out that 5 per cent of the adult population, the population of those more than twenty years old, in Japan at the present time have active tuberculosis.

This is not unique. Dr. DeSilva from the University of Ceylon at Colombo who was here for several days, auditing, tells me that the situation in his country is at least as bad, while some of our discussion here has implied that tuberculosis is almost under control. This isn't so, even if you will remember our circumstances here in this country where the lay press can devote so much space to polio. As Dr. Dubos pointed out in his opening remarks, polio has only about a tenth of the significance in mortality and morbidity, even this year, that tuberculosis has.

When we were first discussing setting up this program, we thought it would be very nice to have a colloquium rather than a symposium. Then it seemed to be frightfully selfish if we asked so many noted people to gather together and talk among themselves "over the flowing bowl." To make it a proper colloquium by permitting others immediate profit as well, and knowing the inevitable lapse of time between discussions and publication, it seemed proper to us that the rest of you who have been here listening and contributing, too, should also be invited.

We are hoping today that we may at least get back toward the colloquium style, even though there is a coffee cup in between which will help, in a facsimile of the bowl, to carry us along.

Just to set the record straight, despite what has been said, the idea of a symposium came about because of Dr. Arthur Robinson's insatiable curiosity. He just couldn't find out enough, and it was Arthur's asking Gardner Middlebrook, "Why can't we do something to learn more?" that really started this off.

During this last morning we hope to accomplish several things. First, I am going to ask Dr. Palmer and Mrs. Ferebee to share with us their interest and experience and then ask Dr. Rosenthal to share some of his enthusiasm for BCG with us. I have told him I am going to interrupt him, too, if necessary, because I intend to be a very hard-boiled moderator. From what individual people have said, they each have three hours of discussion prepared for a three-hour session. I am going to have to cut off some of the discussion, but there are so many important, unanswered questions, both on the part of the participants and of the auditors, that we trust we can get through much of it, and as informally as possible.

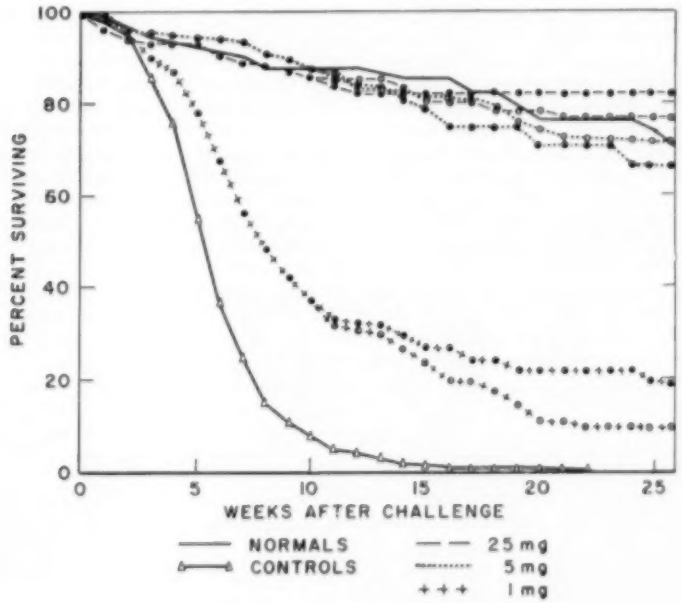
Dr. Palmer is the Director of the Research Program in the Tuberculosis Control Division of the United States Public Health Service. Would you please start off with your comments, Dr. Palmer?

DR. CARROLL E. PALMER (Washington, D.C.): There have been several references to some of the studies we have been doing, and I am glad of the chance to talk informally about them, to show a little of what we have done on the question of the prophylactic use of isoniazid in guinea pigs. And I should like to be fairly specific and show a few slides, even at this late stage of the program.

First, a few words about how the studies were started. About four years ago we began at Atlanta, Georgia, a fairly extensive research program on BCG vaccination of guinea pigs. We were primarily interested in questions of allergy and immunity, and hoped to get information that would be useful for the BCG vaccination programs that are going on all over the world.

I think we have seen indications at this meeting of the kind of difficulty there is about the allergy-immunity question in tuberculosis. On the one hand, some people believe there is no connection at all between allergy and immunity; yet, on the other hand, we go right ahead in vaccination programs using tuberculin sensitivity to select people to be vaccinated, and we also use tuberculin sensitivity to judge whether or not a person has been effectively vaccinated. Having been involved very much with the international BCG vaccination programs, we believed that by taking our field experience and going into the laboratory, we might learn something that would be generally useful. So we began a series of vaccination studies in guinea pigs.

Then, about two years ago, we decided we should try giving isoniazid at the same time as we infected guinea pigs, to see how useful isoniazid might be for prevention—in other words, the prophylactic use of isoniazid. We found out that isoniazid could be given to




Solid circles are used for groups receiving isoniazid continuously; open circles starting after 10 weeks are used for subgroups for which isoniazid was discontinued at that time.

FIG. 1. Percentage of guinea pigs surviving at each of twenty-six weeks after challenge infection with virulent tubercle bacilli.

guinea pigs in their drinking water, and started a batch of 1,200 animals on a project aimed at finding out what happens if you give them isoniazid in the drinking water and infect them at the same time.

The results of that study were so interesting and so promising, at least to us, that we have now turned over the whole Atlanta laboratory activity to an attempt to learn more about the prophylactic use of isoniazid (at the moment) in tuberculosis in guinea pigs.

I should now like to present some of our experimental results. In the graph of figure 1, the vertical axis shows the percentage of animals that survived for different periods after the zero point, which is the time the guinea pigs were challenged with a very strong dose of living, virulent tubercle bacilli a few days after they were started on isoniazid in their drinking water. The horizontal scale is in weeks, from zero to 25.

In this first experiment we included about 1,200 pigs, divided into five groups. One group was the usual kind of control: animals that were challenged but not treated.

We also included another kind of control—a group of animals that were not challenged and were not given the drug. Those we called "normal."

The other three groups were given isoniazid. One group got 1 mg. per kg. of body weight per day; another got 5 mg. per kg. per day; and the third group got 25 mg. per kg. per day. I want to focus attention at the moment only on the first ten weeks. The controls died very rapidly. At the end of ten weeks most of them were dead, which is an indication of the very heavy challenge infection they had received. The group that got 1 mg. per kg. survived longer, but still they died rather rapidly. The group that got either 5 or 25 mg. per kg. survived quite as well as the group of "normals" that were not challenged and received no isoniazid.

At ten weeks we discontinued the drug in half of the animals in each group that had been getting isoniazid. The subgroup that had been getting 1 mg. per kg. and continued to get the drug survived somewhat better than the corresponding subgroup in which the drug was discontinued. For those that got 5 and 25 mg. per kg., discontinuing the drug seemed to make no difference whatsoever. The survival rates are the same for the subgroups in which the drug was continued and for those in which it was discontinued.

Because we had put a sizable number of animals into this experiment, further studies were possible. As shown in figure 2, a second challenge was given fourteen weeks after the original challenge. A new control group was provided by challenging some of the normals that had not been previously treated or challenged. At the same time, we challenged the animals that had been given 5 mg. or 25 mg. continuously and, also, subgroups that had had the drug discontinued ten weeks after the first challenge but without resuming any treatment in these subgroups. It is evident that the group that had previously received 25 mg. daily for ten weeks survived the second challenge better than the controls, but they didn't survive quite so well as those that had previously received the 5 mg. dose. Those receiving the drug continuously survived about as well as the normals.

We interpret these results as indicating that animals surviving a *treated* virulent challenge apparently acquire some degree of resistance to a subsequent *untreated* virulent challenge. Not only that, but, if they get a fairly large dose of isoniazid during the first challenge, their protection against a later challenge seems to be less than if they have had a smaller but still effective dose.

So much for that. I think probably one of the critical questions about which there may be discussion is: Can you call that some kind of resistance or immunity? Actually, the survival curves for this experiment are the same kind that we repeatedly get after vaccination with BCG. In fact, the results are very similar to those obtained with many different vaccines we have used in previous experiments.

We have done some additional studies that I would like to describe quickly.

The question of dosage of drug and the frequency with which you give it seems to be a very important one. Therefore, we set up another large-scale experiment in which we had a series of different doses given at different frequencies. The results showed that a daily dose of 2 mg. per kg. gave a longer survival time than the 1 mg. per kg. dose shown in the previous figure. A dose of 3 mg. per kg. given three days a week was not quite so

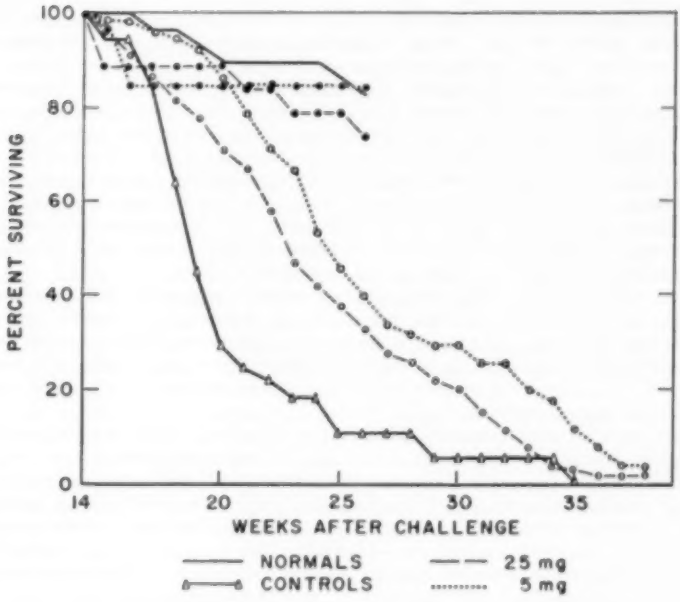



FIG. 2. Percentage of guinea pigs surviving each week after second challenge infection with virulent tubercle bacilli fourteen weeks after first challenge.

effective as 2 mg. per kg. daily, whereas 5 mg. one day a week and 4 mg. three days a week were slightly more effective. In none of those groups, however, did the animals live as long as the normal controls.

The survival curves for all of the other drug regimens were very much alike, and about the same as for the normal controls. Those regimens included 5, 4, and 3 mg. per kg. daily, 5 mg. per kg. three days a week, and 10 mg. per kg. three days a week.

It seems to me that one of the most important and useful bits of information that has come out of these studies is that a dose as low as 3 mg. per kg. daily or 5 mg. per kg. three days a week in the drinking water is apparently as effective as larger doses in keeping the survival times of the guinea pigs as good as normal, unchallenged, untreated animals.

We are going ahead with this experiment in which, at the end of twenty-two weeks, some of the animals are taken off the drug and re-challenged to determine whether or not their acquired resistance or immunity can be improved by varying the dose and the frequency of administration of the drug.

There is one additional point brought out in an experiment in which we studied the effect of the duration of treatment. All groups were getting what seemed to be the lowest but still effective dose, 3 mg. per kg. daily, and in this experiment we stopped the drug after two weeks in one group; after four weeks in another; after six, eight, and ten weeks; and, finally, the sixth group was kept continuously on the drug.

As far as can be determined, 3 mg. per kg. of isoniazid daily for only two or four weeks is not sufficient but, when given for six weeks or longer, the survival curves are as good as in the normal controls.

That is all I have time to summarize. We now have a number of projects going on in

Atlanta. This report, I hope, is an indication of the sort of thing we are doing to see how much protection one can get by starting guinea pigs on isoniazid in the drinking water shortly before they get a virulent tuberculous infection.

DR. ALWAY: Thank you, Dr. Palmer. There is more material than Dr. Palmer has given us so far in their far-ranging interests. Mrs. Ferebee is the deputy director of research for the U. S. Public Health Service. Will you share some more of your observations with us, Mrs. Ferebee?

MRS. SHIRLEY H. FEREBEE (Washington, D. C.): Not so long ago, the studies in diagnosis, prevention, and treatment we were carrying on in the different parts of what the Public Health Service then called the Field Research Branch in tuberculosis were really quite separate activities, but now results from the separate studies are beginning to fit together and show at least the outlines of a total pattern.

I would like to tell you a little about some of our studies in public health research.

In the control studies on the usefulness of BCG vaccination, the initial tuberculin testing allowed us to classify the persons in the study populations according to their reaction to tuberculin. Those not reacting to a dose of tuberculin we regard as specific we called negative. We subdivided the negatives at random into those we vaccinated and those we held as unvaccinated controls.

At this point we set up rosters in order to follow not only those whom we vaccinated and those we held as controls, but also the tuberculin-positive subjects.

The studies were carried out in widely separated geographic locations, representing both high and low tuberculosis morbidity and mortality rates, with different mechanisms in each for finding out about tuberculosis cases and deaths which occur in the study population. Yet, in each of these independent studies the most clear-cut finding thus far is that, in the years since the vaccination program, most of the cases have occurred among those who were not eligible for vaccination, that is, among those who were tuberculin positive at the time of the vaccination program.

The Puerto Rican control study on BCG included children from one to eighteen years of age. A group of 82,000 children were originally tuberculin positive; 27,000 tuberculin-negative children we held as controls, and 50,000 tuberculin-negative children were vaccinated. In the four years since the vaccination program, 577 cases of tuberculosis have occurred among the 82,000 positive reactors, 53 among the controls, and 65 among the vaccinated. This is an annual rate per 100,000 persons of 160 among the positive reactors, 46 among the controls, and 31 among the vaccinated. Although the rates are low for both groups, the vaccinated group had only two-thirds as much tuberculosis as the controls. But the really striking findings are that three-fourths of all of the tuberculosis occurred among those who were already tuberculin positive and were not eligible for vaccination, and that the rate among the positive reactors is about four times as high as among the controls.

In Columbus, Georgia, we carried out a vaccination program in the general population in 1950. There were 31,000 positive tuberculin reactors and approximately 17,000 persons in each of the vaccinated and control groups. In the four and one-half years since then, there has not been much tuberculosis in the study population: 41 cases among the positive reactors, 5 among the controls, and 4 among the vaccinated. Although such small numbers provide no adequate basis for evaluating BCG, there was 80 per cent as much tuberculosis among the vaccinated as among the controls. But three-fourths of the tuberculosis has occurred among the tuberculin-positive group in a study population in which less than half were tuberculin positive.

In a very different population group, we have again found tuberculosis occurring chiefly among the positive reactors. In 1950, in cooperation with the Navy Department, we arranged for the tuberculin testing of a large number of recruits at the San Diego Naval Training Station at the time of their induction into service. As a by-product of the original study, it was possible to set up a name file of 69,000 men. Later, with the assistance of the Committee on Veterans Medical Problems, reports of all cases of tuberculosis that appeared during the period of naval service and, afterwards, in the Veterans Administration

were matched against that file. In the three years since their induction, 30 cases have been discovered among the positive reactors and 37 among the negative reactors. The rate per 100,000 person years is 132 among the positive reactors and 20 among the negative reactors.

We believe that probably the most important finding from these studies is that most of the tuberculosis occurs among those who cannot benefit now from a BCG program. So, if we wish really to do something about those who are most at risk of becoming cases of active tuberculosis, we must do something about the tuberculin-positive group.

We, like many others, had already in our cooperative studies of hospitalized patients become convinced that isoniazid was a very potent drug in the treatment of active tuberculosis. The next step logically was to see how effective isoniazid would be in preventing complications of a primary complex in children. With 32 pediatric centers throughout the country we have undertaken a cooperative study in which half of the children, on a randomized basis, are receiving isoniazid in the range of 4 to 6 mg. per kg. per day for one year, while the other half are receiving a placebo which looks and tastes like isoniazid. We have thus far more than a thousand cases under observation. We hope we will be able to tell at the end of a year's observation whether isoniazid has successfully prevented meningitis and other complications of the primary infection.

The study is carried out in such a way that each physician who is giving the medication does not know whether he is giving to a particular child isoniazid or a placebo. He is free to remove any child from the prescribed medication whenever the condition of that child so deteriorates that he believes specific chemotherapy is definitely required. He makes his decision about whether the child really needs specific chemotherapy before he finds out whether the child has been getting isoniazid or a placebo. After six months of observation on more than 500 cases, only one case of meningitis has occurred. That was in the placebo group. Nineteen children have been removed from the prescribed medication: twelve who were getting the placebo, seven who were getting isoniazid.

The difference between seven and twelve cases, I think you will agree, is not great at this point. There are undoubtedly some differences between individual investigators in what they consider indications requiring a child's removal from the unknown medication. Thus, we have had these cases reviewed after their removal by a group of three participating investigators, who also were ignorant of the nature of the medication. It is interesting that the three who reviewed the twelve placebo cases and the seven isoniazid cases believed that only one case in each group should actually have been removed. The complication in the placebo group is a questionable peritonitis. The question is whether or not the peritonitis is tuberculous. In the isoniazid group, it is Pott's disease, with a question of whether or not it already existed at the time when the child came into the study.

We are, I think, learning a good deal in this study as it progresses, and we may learn whether isoniazid will actually prevent complications of primary complex. However, Dr. Palmer and I both feel that it would be even more important to protect children from having primaries. Perhaps we can make an environment which is safe for our children by using isoniazid to make the active cases noninfectious, and possibly by using isoniazid to prevent the reactivation of a previous infection among the tuberculin-positive reactors. But only time and the most carefully controlled studies will show what can be done.

DR. ALWAY: I would like Dr. Palmer to make one more comment. Did you tell me yesterday some of your observations regarding the current apparent sources of tuberculosis in this state? Will you comment on what is happening in the country at large?

DR. PALMER: I am not sure I know what you mean.

DR. ALWAY: Yesterday you were commenting about the sources of tuberculosis being other than the active, open ones in the Baltimore area and on other studies you have done.

DR. PALMER: The point that Dr. Alway means, I think, is that in connection with this pediatric study we have had some difficulty finding tuberculin-positive children to include in the pediatric program. It was really Dr. Sifontes' notion first to go into the slum areas in Puerto Rico, house to house, to pick up the young children and do tuberculin tests on them, and thereby increase the number of children who could be put in the study.

Dr. Sifontes did this, and he picked up 60 cases for the study and found quite a high

percentage—I believe it was 35 per cent—of the children who were picked up in those really very bad areas who were positive tuberculin reactors.

So, we thought we might do that in other cities in the States to pick up more cases. We went to Savannah, Houston, and New Orleans, and in each of those cities we went into the worst slum areas that we could find, testing more than 1,000 subjects in each city. Only about 2 or 3 per cent of those children were positive reactors, a remarkable finding. It was hardly worth while doing the testing to try to find new cases in these cities.

I think that is another indication of how rapidly tuberculous infection is being reduced in this country. We see it, of course, in many surveys on school children, in which the frequency of positive tuberculin reactors, even in high school students, is down around 3, 4, or 5 per cent. Of course I think that is most important in terms of this Symposium, broadly looked at.

What is happening to tuberculosis infection rates in children in this country? We have come to a new stage: the rates are very much lower than they have been.

DR. ALWAY: Dr. Nelson, I was not being polite when I said you were supposed to do something constructive this morning. I wonder if you would comment first upon this material.

DR. NELSON: I had not intended to do more than ask a couple of questions; but if I may, Carroll, on the part that you presented first, may I say it would be helpful, at least to me, in thinking about these data, if you would answer two questions:

First, did you do tuberculin tests on the guinea pigs in the Atlanta study? If so, would you be able to give us some sort of notion of what the rates of positive reactors were in the various groups?

The second question is this: I think you implied, but I wasn't certain (and it seems to me to have some importance), that in the last slide you showed, in which you were evaluating varying dosage schedules at various times—are those pigs being kept under observation so that you can find out whether or not there are differences that might appear at a later date? In other words, might there perchance be long-range differences in pigs that have had therapy for a short period of time?

On the data that Mrs. Ferebee presented in relation to the results of BCG vaccination, some questions came up. The one question that to me seems most obvious is this: What did you know about the status, not tuberculin-wise, but tuberculous-wise, of tuberculin-positive children? Did those children who had positive tuberculin reactions have adequate examinations, and could you at that time identify the amount of tuberculosis they had?

I think the implication is obvious that, if perchance in that group you had children who had active disease and in whom there was a potential of its being progressive (may I say that, Dr. Terplan?), that factor alone might be responsible for much of the difference.

Second, it would be important to know whether or not those children who were tuberculin positive did or did not, in significant numbers, continue to live in contact with tuberculosis.

Of course it would be nice to know, but I suspect it is relatively impossible to know in your kind of study, what the chances or probabilities were for infection in your vaccinated and control groups, but there I think we would have to lean on numbers, and assume that whatever variations there might be for individuals would be ironed out for the group.

Those are the questions that come to my mind. I suspect that most people will at least agree that it is important to have these data given to us directly.

DR. ALWAY: In attempting to keep this as informal as possible, we might answer the questions as they arise, one by one. Will you answer those first, Dr. Palmer?

DR. PALMER: My friend Nelson wants to know if we tuberculin-tested the pigs. I could hardly be involved in any kind of tuberculosis study without doing tuberculin tests. We certainly tuberculin-tested the animals, roughly at four-week intervals.

I can't get into this matter of the results of the tuberculin sensitivity in this experimental work except to say that the higher the dose of drug, the lower the level of tuberculin sensitivity produced. You can give enough isoniazid essentially to prevent tuberculin sensitivity; but at the lower doses you certainly do get tuberculin sensitivity.

That has to be measured, I think, not in terms of the usual positive-negative, but in terms of an analysis of the distributions of the sizes of the reactions according to the dose. I just don't believe we can contribute more now to that question of the tuberculin sensitivity, but we have a tremendous amount of material that is presently being analyzed.

Concerning the other question of whether we are keeping these animals for long-time observations: Yes, we are. On some of the experiments we are taking parts of the various groups and are doing second challenges, treated and untreated, and all that kind of thing; but one group in particular that I showed this morning, those animals that had had the drug two, four, six, eight, and ten weeks and continuously—that group we expect simply to hold and see what is going to develop in later months.

Mrs. Ferebee: With respect to the question of what we know about the tuberculous status of these populations at the time of the vaccination program, I think we have to say that in Puerto Rico we knew only that they were school children who were healthy enough to be in attendance at school. We did not have X-rays on them. They had not been identified and removed from school by any case-finding program operating in the Island. I think we really have to say that we simply knew nothing about these children except their tuberculin status.

However, in Columbus, Georgia, where the results are very much the same, we had done a very careful X-ray survey, and we had an X-ray for each person in this population. The results here are confined to those who were X-ray negative, positive reactors with nothing at all on their X-rays. When a case of tuberculosis developed in the subsequent period in that group, we went back and read the initial X-ray again. If it appeared, as it sometimes does with even the best X-ray reading, that we saw something that we realized we had missed, we put that case aside and didn't count it. We counted it as having essentially existed, although we weren't smart enough to have recognized it at the time.

The group of Navy recruits in whom vaccination itself was not a question, but in whom there is this tremendous difference between the amount of tuberculosis developing in the positives and the negatives, that group was very carefully screened, and they had X-rays which were completely free of pathology at the time of their induction. In the same way as in Columbus, Georgia, X-rays taken in cases arising in that group have been re-read against the initial X-rays, so that any case that was missed has been taken out of the study.

I think we can say with some certainty that in the two populations the tuberculosis occurred since our initial observation.

Dr. Nelson, we don't know anything about whether the people who were tuberculin positive were so because they lived in an environment where they could have contact with the tubercle bacilli and then continued to have contact in either Puerto Rico or Columbus. However, that is the beauty of the Navy material in that all of these boys were tuberculin tested at the time of their induction, and then their environment was artificially changed and made the same for the tuberculin-negative and -positive subjects.

Dr. Kassowitz: I would like to make two very brief comments, and with your permission show three slides.

I want to make a comment about the disappearance of positive reactors in this country. I have already published a little survey of the last ten years in a children's hospital dispensary in Milwaukee, Wisconsin, where even ten years ago, from 1945 up to 1955, the incidence among the lowest economic group, which is an industrial type of population, was not higher than 5 per cent.

This was a selection of tuberculosis suspects in a very crude way, as judged by the resident or attending physician in charge, on the basis of suspicious X-ray shadows. The tuberculin tests were done only on those suspicious cases.

In this selected material, in which there is negative selection, we didn't find more than 5 per cent ten years ago, and this dropped to 3 to 3.5 per cent at the present time, which includes quite a number of recent immigrants from Puerto Rico who have a high incidence of positive tuberculin reaction.

The point I want to make is that the reactors are disappearing. The cases of meningitis

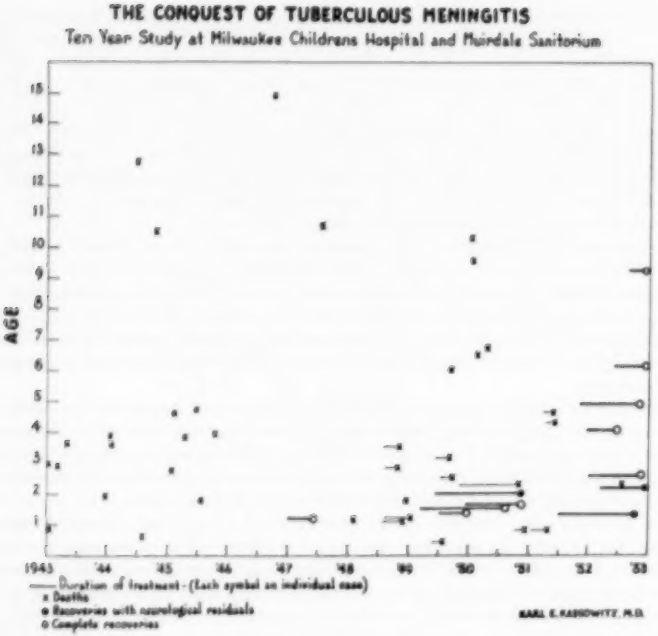



FIG. 1.

are still with us, not in great number, but in too great a number. What we have heard from Scandinavia during the last several days, and what we read in the literature, is that reactors are mounting due to BCG vaccination. In these countries the tuberculin test has to be considered of no diagnostic value any more, due to mass BCG vaccination. But meningitis and other hematogenous tuberculosis, according to those reports, have practically disappeared. This is the situation that I think we are dealing with and is what other participants have mentioned in detail.

I would like to show three slides which illustrate the results of a survey of meningitis and miliary tuberculosis during the last ten years. In the first slide (figure 1), the crosses mean death from meningitis after admission to the hospital; the horizontal line is the duration of stay in the hospital. The circles indicate survival without neurologic residuals. The black dots mean survival with any degree of neurologic residuals.

I think that, while this is not as large a group as Dr. Lincoln was able to report on, this method is a rather descriptive way of showing results. We see a survival rate of more than 90 per cent at Milwaukee County Hospital and the County Sanatorium.

In figure 2 we have a few scattered cases of miliary tuberculosis without meningitis. There were only four fatal cases.

The case described in figure 3 represents one of the rare instances of active primary tuberculosis originally treated in 1953, wrongly, with streptomycin and PAS, with very good results, which cleared on X-ray. After seven months (months 5 to 12 in figure 3), meningitic signs appeared. Cell counts are shown in the graph. Treatment was continued. Isoniazid was added. Culture of cerebrospinal fluid showed isoniazid resistance. Pyrazinamide was added. There was complete recovery. This is an important and very rare case, I am sure, in which, due to bacterial resistance, meningitis developed. We don't have to give up if we use the drugs to which the bacilli show susceptibility *in vitro*.

## CASES OF MILIARY TUBERCULOSIS WITHOUT MENINGITIS

### 10 YEAR STUDY AT CHILDRENS HOSP AND MUIRDALE SANATORIUM

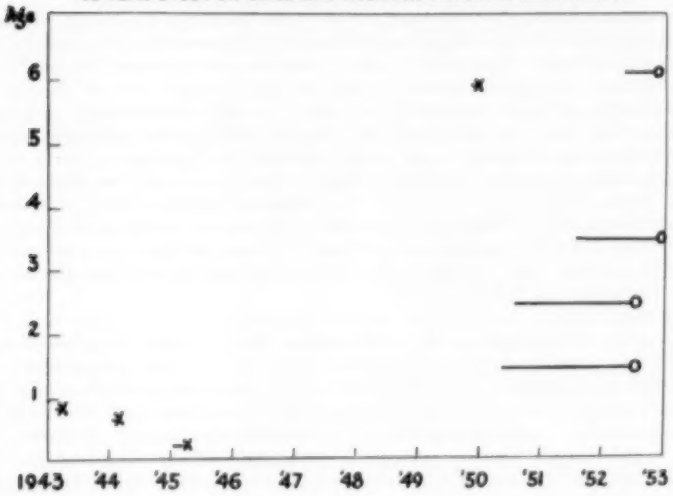
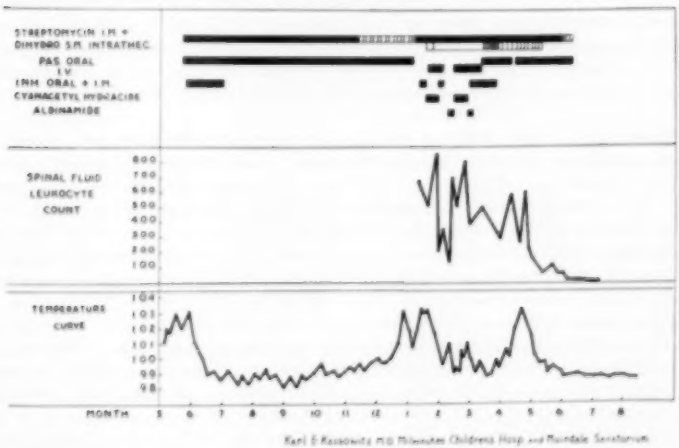



FIG. 2.

### A CASE OF PARADOXICAL TUBERCULOUS MENINGITIS

SHOWING THE PHENOMENON OF BACTERIAL RESISTANCE AND ITS CONQUEST

A 3 year old boy on intensive 7 months SM + PAS therapy for active pulm. tb.; asymptomatic, afebrile, X Ray clearing. Sudden hyperpyrexia, meningismus, positive cultures from spinal fluid. Therapy: daily SM, 25 intrathecal SM, I.V. PAS for 30 days, then continuous oral PAS, INH i.m. + oral until bacterial resistance shown in vitro. SM completely sensitive, 1-14 and 2-11. PAS partially resistant. Short term courses of Aldinamide and Cyanacetyl Hydracide added. Very slow but steady response to intensive SM + PAS medication. Complete recovery 13 months after start of tet., 6 months after onset of meningitis. No residuals except 8th nerve damage.




Karl E. Kassowitz, M.D. Milwaukee Children's Hosp. and Muirdale Sanatorium

FIG. 3.

DR. CANETTI: I would like to go back a little to the absolutely remarkable experiments which Dr. Palmer and Mrs. Ferebee have presented. I feel uncomfortable. While I deeply admire the experiments, I have to pick out in them a point which perhaps needs a little more clarification, and that is this very central point upon which we have already talked a lot in these last few days, namely, the problem of whether chemotherapy, more or less prophylactic, may build up some resistance—if, after stopping chemotherapy, there may remain something like immunity or increased resistance in the animal.

I must consider one of the slides Dr. Palmer showed us, showing the results of second challenge. I must first review my position. You gave your animals a heavy infection. You treated them with 25 mg. doses of isoniazid, and you had a very high survival rate. After a certain amount of time you gave them a second challenge. Without any treatment, these animals withstood this infection better than the controls, and so on. They showed a good degree of resistance; am I right?

DR. PALMER: Yes.

DR. CANETTI: My interpretation would be that the good resistance of these animals was simply due to the heavy dosage of your first infection. You gave these animals a first infection with a good number of bacilli. What did you do by doing that? You injected into them a good amount of immunizing antigens, without having any great multiplication of the bacilli because you gave them isoniazid. Yet, you placed in them a sufficient dosage of antigens to give them a significant degree of immunity.

So, the immunity you observed was not in any way related to your chemotherapy, but was related to your very first challenge.

You, of course, will answer: If that is the case, it would be the same in human beings; we would observe the same results by giving them this kind of chemotherapy. Now I think that is just the point. In human beings you would not observe the same result. Why not? Because the first infection given to your guinea pigs was a heavy one. You wouldn't observe that degree of infection in human beings on chemoprophylaxis. Human beings would be infected by one, 10, 50, or 100 bacilli, and your chemoprophylaxis would immediately stop the multiplication of these bacilli, which means that, in these human beings, there wouldn't be antigens enough to build up something like good immunity. So what would probably happen in the human beings—I say probably because I think we would expect it—they would have no immunity at all or so little immunity that it would be the same as no immunity.

I think your very remarkable experiences, which I admire greatly, would perhaps be interesting to repeat, setting them up in another way. Challenge your guinea pigs with a very small dosage the first time and treat them, and then see if after having infected them with 10 or 100 bacilli and treating them, you would have, after you stop the treatment, anything like an immunity or a protection. This, I think, would bring us nearer to human conditions.

DR. MIDDLEBROOK: Dr. Schmidt did that with monkeys under more or less natural laboratory conditions, and observed that 50 per cent of the animals subsequently developed tuberculous disease, even though they were removed from the infective environment.

DR. CANETTI: Dr. Schmidt's animals were not challenged by superinfection.

DR. MIDDLEBROOK: They didn't have to be. They presumably got spontaneous tuberculous infection by natural exposure during administration of isoniazid. This is the point.

DR. CANETTI: Well, we are now speaking about Dr. Palmer's and Mrs. Ferebee's guinea pigs.

DR. ALEXANDER: I thought Dr. Middlebrook would ask this question. Since he hasn't, I would like to raise the question whether the guinea pig is a good animal, for this reason: It is impossible to detect in guinea pigs the most frequently occurring variety of isoniazid-resistant organisms, namely, the catalase-negative ones, because these have no true pathogenicity for guinea pigs. I would like to have him elaborate on that.

DR. MIDDLEBROOK: I just want to point out in a manner—I won't be too sophisticated—but a manner which reflects how much experimental work you have to do before you can

draw conclusions for public health work. If you take the H37Rv strain and try to isolate from that strain a catalase-positive, not so highly attenuated, isoniazid-resistant mutant, you can't get one. We have been unable to get one. Whereas, if you take another strain, which we have in the lab, the Pearson strain, you readily get such a mutant. Now, Dr. Palmer, if you had used the Pearson strain for challenge of your guinea pigs, you would probably, with the size of inoculum you used, have had enough catalase-positive, isoniazid-resistant mutants so that you would have gotten more bacterial multiplication during isoniazid treatment. The whole picture might be different just as a result of a difference in the particular strain that you used.

You see, there is no such thing as "the tubercle bacillus," as I suggested yesterday. There is no such thing as "the" tubercle bacillus, and it seems to me that Dr. Canetti has pointed out very clearly for you the basic concept involved in the problem of chemoprophylaxis.

Now, aside from that, the relationship between the size of the dose of the drug and the degree of immunity manifested later certainly seems to me to have resulted in very interesting differences.

I am fascinated because here again, as in Dr. Schmidt's monkeys, the differences are easily explained by a difference in drug dosage.

In general, in human beings, of course, dosage doesn't make any difference, or so it says in the literature: "150 milligrams is adequate per day for adults," but in monkeys and guinea pigs the dose does seem to make a real difference!

DR. RIST: I should like to go back to the immunologic research of Mrs. Ferebee and ask a question which I am sure is on the lips of many of the attendants.

She seems to have been surprised by the fact that the initially positive tuberculin reactors had greater risk of having later tuberculosis than the nonreactors, and I didn't quite understand her surprise. I suppose she referred to the classical experiments of Heimbeck showing that the risk of reactors was less than the risk among nonreactors, but the conditions were not at all the same. In the Heimbeck experiment all the youngsters were put in a dangerous environment. That's a medical school, a hospital. I suppose in the Navy, for instance, the environment is quite different, so that the risk of the reactors may be much greater than the risk of the nonreactors when you put them in an environment where there is less chance to get any tubercle bacilli than, for instance, in a hospital.

Did I well understand your surprise?

MRS. FEREBEE: I think the BCG programs would appear to hold out hope for so many countries on the basis that, if one protected the negatives, one had effectively attacked the problem. That would lead one to think that, in general, it is the negatives who are at greatest risk.

In addition to the studies that you have cited, a number of others have shown that more tuberculosis did, in fact, occur among those who were tuberculin positive.

DR. MITCHISON: I would like to make this comment on Mrs. Ferebee's and Dr. Palmer's animal experiment. There would seem to be three distinct ways in which immunity might have arisen to the second challenge.

The first of these has been suggested by Dr. Canetti—the bacilli, in fact, were present in large dosage, but did not multiply. On the other hand, one has to remember that ultraviolet-light-killed vaccines have to be given in enormously high doses. Therefore, this doesn't seem to be a probable explanation. The second possibility is that the immunity is caused by the production of isoniazid-resistant mutants multiplying to some extent in the animal body. And the third possibility is that there may have been persistent drug-susceptible bacilli in the animal tissues at the end of the first period of treatment which produced a very low-grade chronic infection capable of initiating and maintaining the degree of immunity which was subsequently manifested.

I think the second and the third possibilities could be quite easily distinguished experimentally, and this is a most important next stage in your studies.

DR. MCDERMOTT: It seems to me that we are really talking about two entirely different

sets of host-parasite relationships here when we are talking about the Palmer-Ferebee experiments in guinea pigs, on the one hand, and the treatment of people who have recently become cutaneous reactors to tuberculin, on the other. There is grave danger that the two sets of things, which are really quite different, may get mixed up in our own minds, and in everybody else's mind as well, and so, Dr. Alway, I crave your indulgence, to try, if I may, to pinpoint the problem.

Let us take the human problem first. We have the human being who has become a cutaneous reactor to tuberculin. We know that a certain amount of acquired resistance occurs in the human at some time after he becomes infected.

We know, as Dr. Dubos said, that this may not be worth having because of the price one has to pay in the future possibility of metastatic lesions. There is, nevertheless, a certain amount of acquired resistance which occurs following tuberculous infection. The big point, it seems to me—and this is the crux of the situation of the clinician—is that by the time the patient has become a cutaneous reactor to tuberculin, has everything happened that is going to happen in terms of the acquisition of resistance? Or are there increments of host resistance which can occur after the time when cutaneous sensitivity has appeared—increments which could be prevented by the administration of antimicrobial therapy? Now, whether that is so or whether that is not so, at this stage of our ignorance, is strictly a bet.

It is my own bet, Mr. Chairman, that by the time a patient has become a cutaneous reactor to tuberculin all that is going to happen in terms of the acquisition of host resistance has already happened, and that the introduction of antimicrobial therapy at that point will not significantly prevent the development of host resistance. It will, of course, do the additional things of treating the primary lesion, preventing metastases which have not already occurred, and treating metastases which have already occurred.

The Palmer-Ferebee situation is an entirely different situation. It is an important situation and their experiments are really very beautifully done. I think it is a grave mistake to sort of make Palmer and Ferebee responsible for what happens in the human and *vice versa*.

The Palmer-Ferebee work has relevance in two ways: (*1*) It constitutes "beginning experiments" which were properly done before all of the subsequent experiments which everybody has outlined, ringing the changes, et cetera; and (*2*) in those circumstances, which I happen to believe are very few in the world, when one might want to give isoniazid to people who do not have tuberculosis in order to prevent them from getting tuberculosis from their associates who are highly infectious.

The guinea pig experiments would have some relevance in that situation, and it is that very sort of thing that Dr. Schmidt's experiments showed did not look very good in the monkey; and I believe on theoretic grounds would not work any better on human beings. So, I could return to where I started, Mr. Chairman, and say that, while I believe the Palmer-Ferebee experiments are very important within their field, I believe that that type of chemoprophylaxis will probably not work in humans. I believe the big point and the big question which we have to decide is: Does antimicrobial therapy interfere with anything once the patient has acquired tuberculin hypersensitivity?

DR. LACK: Dr. Nelson asked Mrs. Ferebee whether those who were tuberculin positive showed a higher incidence of active tuberculosis at the time of vaccination, and Mrs. Ferebee said that only in the Navy group had adequate screening been done. This appeared to refer only to the lungs.

I just want to point out that in an examination of 30,000 consecutive tonsillectomies, 22.8 per cent of the tonsils showed some evidence of tuberculous disease. So it seems to me that you have a possible source of existing infection which might have been overlooked.

DR. PLATOU: I think clinically we all know it is difficult to generalize and each case must be highly individualized, but relative to Dr. McDermott's, Dr. Palmer's, and Mrs. Ferebee's contributions, I wonder if it wouldn't be pertinent to suggest some discussion on the interval of therapy, the duration of therapy, designed to suppress complications.

We have taken an interval out of thin air based on the usual time during which meningitis develops, and on the usual time for healing of pulmonary lesions. This is generally used around the country, and perhaps around the world, for determining duration of therapy of all forms of the disease.

I wonder if it wouldn't be better to go on record and suggest that we discuss some period, perhaps six to twelve weeks, beyond suppression of clinical evidences of active infection, be they in bone, in nodes, in meninges, or wherever, as a point at which therapy may be stopped. Whether this is prophylaxis or whether there is active disease at the time of initiating chemotherapy, shouldn't we take some interval beyond the period of active disease rather than start from the zero point at which therapy is started.

I think there is a tremendous confusion around the country on this point. When do you let loose the bull's tail?

DR. LINCOLN: I think there is obviously no rule. I think you have to assume that, if you are going to try to prevent complications, you have to treat for the period of time during which complications are most likely to develop. Then I think we have to wait for the accumulation of evidence such as we are beginning to get now: that meningitis, for instance, has been seen to develop after the short periods of isoniazid treatment and that one would want to go, at least, beyond that period.

If I understand Dr. McDermott's comments, and I am not sure I do, I would want to continue for the present to use a year as the minimum period of treatment until we have better evidence to the contrary.

DR. ALWAY: Dr. Platou suggested this short period of six to twelve weeks, and Dr. Lincoln has suggested a year. Dr. Debré, would you take a specific stand somewhere on this point? Would anyone else?

DR. ALEXANDER: Not a stand, but I would like to ask a question. Does not the duration depend upon how many agents you are using and which ones?

DR. SIFONTES: In regard to what Dr. Lincoln has said about the year, the first group of patients we studied with primary tuberculosis was placed on isoniazid and PAS, including controls. We treated for only four months. The study lasted for a year. After a follow-up study for a whole year in that group we did have patients who had a reactivation of the disease after we discontinued therapy; so I think, on the basis of that limited experience, that it is indicated that therapy should be administered for at least a year.

In regard to Dr. Palmer's and Mrs. Ferebee's story, I would like to make a comment. We are tremendously excited about the study, and we think it is an extremely important study in guinea pigs that points the way to further tests in children. As I say, I think you just have to try it on a large scale in children. It is an extremely important thing but, until that is done, I think we can talk all we want and we will never know anything.

DR. SCHMIDT: I am not going to rise to the defense of the monkey, but there are comments I wanted to make which were brought out before I heard Dr. Sifontes' words.

There have been a number of differences alluded to in the results obtained by Mrs. Ferebee and Dr. Palmer, on the one hand, and by ourselves, on the other. I simply want to point to another difference in the patterns in which our studies were performed. That is the portal of entry of the tubercle bacilli.

I am not at all certain that the portal of entry has any influence on these end results. But the portals of entry were totally different, and I think it would be well to question whether the acquisition of bacilli through the respiratory passages, as probably occurs in many human infections, gives the same sort of result as the introduction of tubercle bacilli subcutaneously or intraperitoneally.

DR. LACK: There is very little information on this question of the susceptibility of man to infection by routes other than the respiratory, but there are some people who have injected themselves with tubercle bacilli in an attempt to commit suicide, and accidents occurred, for instance, in our own hospital when we have unwittingly used tuberculous bone in doing bone grafts in several children.

The one thing that impressed me about studying these cases is that the infection is

invariably mild. For instance, the children who had tuberculous bone grafted had no extension of the disease. These people who have given themselves injections have not developed the extensive disease that one would expect, in spite of massive injections.

DR. ALWAY: I think in our discussion this morning I might be permitted to make one comment, and that is that still, with all this talk about the recent converter, we are not treating skin tests; we are really treating tuberculous lesions. This orientation should be stressed.

We will now have three presentations without discussion.

First, Dr. Sol Rosenthal, Director of the Tice Institute at the University of Illinois, will make some remarks on BCG. Dr. Rosenthal.

DR. ROSENTHAL: I am sure that all of us realize that prevention of a disease is the best way to "control" it. As you know and have heard all this week, once the organism gets into the body we have our complications. We must then talk about treatment. As Dr. Palmer and Mrs. Ferebee showed, cases come from these so-called positive reactors, those who have already been infected, and so we believe that great emphasis should be placed on methods of active prevention. One of these methods, of course, is BCG.

I don't mean to say that BCG should be considered alone. We must use other methods of control as well. I will try to point out this morning that BCG alone is not enough, but that it does help in prevention of tuberculosis.

* * *

[The remainder of Dr. Rosenthal's discussion is presented in the following paper, which was very kindly submitted by Dr. Rosenthal.—*The Editor*]

# THE ROLE OF BCG VACCINATION IN THE PREVENTION OF TUBERCULOSIS IN INFANCY AND CHILDHOOD

SOL ROY ROSENTHAL[1]

## RESISTANCE AGAINST VIRULENT TUBERCULOUS INFECTION

A strictly controlled study of BCG has been in operation in Chicago for more than eighteen consecutive years. Some 21,072 infants and children are involved Approximately half of these children were vaccinated, and the remainder were not vaccinated. Both groups were selected and followed in a similar manner. BCG vaccination, when employed as part of a tuberculosis control program, reduced the morbidity and mortality from tuberculosis from 68 to 100 per cent as compared to the controls. These favorable results have been maintained or improved over the years.

Four strictly controlled studies were carried out in infants and children. The exact method of study has been given in the previous reports. Briefly, the mode of study and follow-up as well as results obtained follow:

*Newborn Infants with No Contact with Tuberculosis* (Program Initiated in 1937)

Newborn infants in the Cook County Hospital were vaccinated on the second to the fifth day of life. It was first ascertained that there was no tuberculosis in the immediate family. This was done at the onset of the study by roentgenographic examination of all members of the household but, in recent years, only the mother is examined roentgenographically, and the tuberculous status of the remainder of the household is obtained by questioning. Although there was no known tuberculosis in the household, these children returned to an environment in which the incidence of tuberculosis was very high. This was attested by the fact that in control children, living under similar conditions, 4 per cent became tuberculin positive at eight to twelve months of age, and 30 per cent were positive at four and one-half to five years. At first the vaccinated and control subjects were alternated so that the number was equal, but later approximately 2 newborn infants were vaccinated (with fresh or dry vaccine) to one control. This accounts for the differences in the number of vaccinated and control subjects. The infants were followed semiannually in the clinic with tuberculin tests, roentgenograms, and physical examination. Approximately 90 per cent of this population was Negro. There were 9,554 children in this group.

There was a total of 18 cases diagnosed as tuberculosis in the vaccinated group as opposed to 63 cases in the control group. The morbidity rates per thousand were 3.3 and 15.3, respectively. There were 2 deaths from tuberculosis among the vaccinated and 8 among the control subjects, constituting mortality rates of 0.4 and 1.9, respectively (table 1). The ratio of morbidity rates of the vaccinated to the control group is 1:4 and that of mortality rates is 1:5.

[1] Institution for Tuberculosis Research of the University of Illinois; Chicago Municipal Tuberculosis Sanitarium; Research Foundation, Cook County Hospital, Chicago, Illinois.

TABLE 1

RESULTS OF STRICTLY CONTROLLED STUDIES OF BCG VACCINATION IN ALL AGE GROUPS CARRIED OUT OVER THE PAST EIGHTEEN YEARS

| | Vaccinated | | | | | Nonvaccinated | | | | | Per Cent Reduction in Vaccinated Group* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number in Study | Tuberculosis Cases | Morbidity Rate† | Tuberculosis Deaths | Mortality Rate† | Number in Study | Tuberculosis Cases | Morbidity Rate† | Tuberculosis Deaths | Mortality Rate† | Morbidity | Mortality |
| Infants | | | | | | | | | | | | |
| Noncontact | 5,426 | 18‡ | 3.3 | 2‡ | 0.4 | 4,128§ | 63 | 15.3 | 8 | 1.9 | 78 | 79 |
| Contact | 311 | 4 | 12.8 | 1 | 3.2 | 250§ | 10 | 40.0 | 4 | 16.0 | 68 | 80 |
| Children | | | | | | | | | | | | |
| Orphanages | 2,370 | 0 | | 0 | | 1,348‖ | 12 | 8.9 | 0 | | 100 | |
| Housing project | 947 | 0 | | 0 | | 944§ | 3 | 3.2 | 0 | | 100 | |
| | | | | | | 875‖ | 3 | 3.4 | 1 | 1.1 | 100 | 100 |
| (Ida B. Wells) | | | | | | 584¶ | 3 | 5.1 | 1 | 1.7 | 100 | 100 |
| Housing project (Altgeld) | 2,574 | 0 | | 0 | | 1,900‖ | 0 | | 0 | | | |
| | | | | | | 612¶ | 0 | | 0 | | | |
| Adults | | | | | | | | | | | | |
| Student nurses | 269 | 2 | 7.4 | 0 | | 281§ | 5 | 17.8 | 0 | | 58 | |
| | | | | | | 476‖ | 3 | 6.3 | 0 | | −17 | |
| Medical students | 472 | 0 | | 0 | | 313§ | 3 | 9.6 | 0 | | 100 | |
| | | | | | | 1,079‖ | 4 | 3.7 | 0 | | 100 | |
| | | | | | | 279¶ | 5 | 17.9 | 0 | | 100 | |

* Rate for control group taken as 100 per cent.

† Rate per 1,000.

‡ One infant had contact since birth with "open" tuberculosis; the other, a "no take" after vaccination.

§ Negative tuberculin reactors.

‖ Positive tuberculin reactors.

¶ Tuberculin reaction status incomplete.

*Newborn Infants in Tuberculous Households* (Program Initiated in 1940)

These newborn infants were derived from various hospitals in the city of Chicago. They were separated from their tuberculous contacts at birth and kept in foster homes for periods up to five months, depending upon the relationship and the severity of the source case. If the mother was tuberculous, there was a period of one to three months of separation before vaccination, at which time a Mantoux test was done. A roentgenogram of the infant's chest was obtained if the mother had tubercle bacilli in the sputum. If any other family member was tuberculous, vaccination was performed at birth and the separation was continued for six to eight weeks. If the source case was considered "closed," the children returned home six to eight weeks after vaccination. This occurred approximately 80 per cent of the time. The control and vaccinated groups were strictly alternated from a master chart in the central office (except at the onset of the study when details of separation were being worked out) and were isolated

TABLE 2

LESIONS SEEN ON ROENTGENOGRAMS OF CHILDREN WITH NONFATAL TUBERCULOSIS

| Diagnosis of Lesions | Vaccinated | | | | Controls | | | |
|---|---|---|---|---|---|---|---|---|
| | No Tuberculous Contact | Tuberculous Contact | Total Cases | | No Tuberculous Contact | Tuberculous Contact | Total Cases | |
| | | | Number | Per Cent | | | Number | Per Cent |
| Lobar | 0 | 0 | 0 | 0 | 4 | 1 | 5 | 9 |
| Parenchymal infiltration | 2 | 0 | 2 | 10 | 10 | 1 | 11 | 21 |
| Hilar infiltration and extension | 2 | 0 | 2 | 10 | 3 | 1 | 4 | 8 |
| Hilar infiltration | 6* | 1 | 7 | 37.5 | 23† | 2 | 25 | 48 |
| Hilar lymph nodes | 2† | 0 | 2 | 10 | 1 | 1 | 2 | 4 |
| Calcification as first lesion seen | 4* | 2 | 6 | 32.5 | 5* | 0 | 5 | 10 |
| Total | 16 | 3 | 19 | 100 | 46 | 6 | 52 | 100 |

* Two patients reacted to histoplasmin.

† One patient reacted to histoplasmin.

in a similar manner. There were 561 cases followed over a period of fifteen years. There was a reduction in the morbidity rate of 68 per cent and in the mortality rate of 80 per cent among the vaccinated as compared with the control groups (table 1).

*Newborn Infants—Both Groups*

There was not only a suppression of the morbidity and mortality from tuberculosis in the vaccinated contact and noncontact infants but, when virulent organisms did gain entrance in the vaccinated host, the lesions produced were less extensive as noted by roentgenography (table 2).

*Deaths from tuberculosis:* Of the 12 control subjects who died, 8 had tuberculous meningitis. Of the 3 vaccinated subjects who died, only one had tuberculous meningitis, and this was a patient in whom the patch test was negative six months after vaccination. Thus, the vaccination in this infant is considered as a "no take." There were 8 deaths in the controls in the first two years of life and 2 deaths among the vaccinated. Of the latter, one infant was exposed to "open" tuberculosis during the first and second weeks of life. The second case was a "no take" following vaccination as described above.

*Cases of tuberculosis in first two years of life:* There were 18 cases of tuberculosis, excluding deaths, in the first two years of life among the control subjects and only one case (two years old) among the vaccinated subjects. It may be concluded that, under the conditions of this study, BCG prevented tuberculosis in the first one and one-half years of life in successfully vaccinated infants.

*Children in a Federal Housing Project* (Program Initiated in 1942)

The entire population of a housing project (Ida B. Wells) situated in the midst of a high prevalence area for tuberculosis was surveyed by roentgenography. The children up to twelve years of age were tuberculin tested, using a

Vollmer patch test, with a subsequent Mantoux test if the patch test was negative. The negative reactors were vaccinated either with BCG or a saline placebo. In each family, the oldest member who was negative to tuberculin was vaccinated, and the other members were alternated as strictly as was possible. At yearly intervals the same procedure was repeated, that is, mass roentgenography of the entire population and tuberculin testing. This program has been in continuous operation for thirteen years. The population is entirely Negro. There has not been a case of tuberculosis among the vccainated children. On the other hand, there have been 9 cases of tuberculosis among the nonvaccinated, which include tuberculin-negative and -positive reactors as well as those in whom the tuberculin reaction status was incomplete. (Two of the 9 died from tuberculosis.) There were 3,350 children followed in this study (*see* table 1).

In another Negro housing project, Altgeld Gardens (progam initiated in 1946), all schoolchildren in grade and high school who failed to react to the Vollmer patch test or to 1 mg. of OT were vaccinated with BCG. Thus, the only controls were positive reactor controls. There were 5,086 children in this group, about half of whom were vaccinated. No cases of tuberculosis in either group have developed over an eight-year period.

*Orphanages* (Program Initiated in 1944)

A roentgenographic survey was made of the entire population of two orphanages. The population included the teachers, the janitors, the cook, et cetera. The persons with active tuberculosis were removed. All of the children were tuberculin tested with two strengths of tuberculin (1:5,000 and 1:100 dilutions of OT). The children varied in age from two to approximately eighteen years. All negative reactors were vaccinated. At yearly intervals roentgenographic examination of the entire group was repeated and the originally negative reactors were retested with two strengths of tuberculin. Over a period of eleven years there have been no cases of tuberculosis among the vaccinated. Among the control subjects who reacted to tuberculin, one active case of tuberculosis and 11 calcified primaries were demonstrated by roentgenography. All of the films had been normal at the onset of the study. Many of these children come from homes in which there is tuberculosis and return there for visits on week ends. There were 3,718 white children in this study (*see* table 1).

*Role of environment:* The highest morbidity rate was found in newborn infants whose exposure to tuberculosis was the greatest, that is, those with tuberculosis contacts in their homes. This morbidity is high irrespective of the fact that the housing, nutrition, and social status were of a higher order than that of the infants in the noncontact groups. Vaccination reduced the morbidity 68 per cent in this group as compared with control subjects who lived under similar conditions. Improvement of housing favorably affected the morbidity rate in children not vaccinated and in nonreactors to tuberculin: 3.2 per 1,000 in contrast to 15.3 per 1,000 in poor housing areas. Complementing good housing with a complete tuberculosis control program, including BCG vaccination, lowered the morbidity to zero, as was found in two Negro housing projects. In two orphanages (white) a comprehensive tuberculosis control program (including

BCG vaccination), and where housing and nutrition were good, likewise reduced the morbidity rate to zero.

### METHOD OF VACCINATION

The multiple puncture disc method of vaccination produces macular-papular to papular lesions. The height of the reaction is four to eight weeks following vaccination. Ulcer formation or local abscesses practically never occur in infants vaccinated at birth or in children, provided the recipients do not react to 1 mg. of OT (100 TU) or the equivalent. In 5,426 infants vaccinated at two to three days of age, only one developed suppuration of a regional axillary lymph node, which healed spontaneously. The local lesions regress rapidly and are usually not visible three to four months after vaccination, leaving no scar.

### TUBERCULIN "CONVERSION" (COMPARING FRESH AND DRY VACCINE)

From previous studies it had appeared that the tuberculin "conversion" in infants occurred sooner for the fresh vaccine (twenty-nine days) than for the dry vaccine (fifty-eight days). In recent studies, with improvements in the methods of cultivation, harvesting, preservation, and increased potency of the strain plus larger numbers of infants studied, the difference in rapidity of the tuberculin "conversion" between fresh and dry has been negated. For example, after five to six weeks, 96 per cent of the infants vaccinated with the fresh vaccine and 97 per cent of those vaccinated with the dry vaccine had positive tuberculin reactions (Vollmer patch and/or 0.1 to 1 mg. of OT). A total of approximately 2,000 infants was compared (table 3). The mean diameter of the tuberculin reaction (1 mg. OT) five to six weeks after vaccination for the fresh vaccine was 13.8 plus-minus 4; and for dry vaccine, 13.9 plus-minus 3.9. Nine weeks after vaccination with dry vaccine, 84.5 per cent reacted to 0.01 mg. OT (mean diameter = 10.4 ± 4.7) and 100 per cent reacted to 1 mg. OT (mean diameter = 18.5 mm. ± 5).

TABLE 3

RAPIDITY OF TUBERCULIN CONVERSION* IN INFANTS VACCINATED AT BIRTH (MULTIPLE PUNCTURE) WITH FRESH OR DRIED VACCINE

| Weeks | Fresh Vaccine | | Dried Vaccine | |
|---|---|---|---|---|
| | Number Tested | Per Cent Positive | Number Tested | Per Cent Positive |
| Less than 1 | 9 | 11 | 7 | 43 |
| 1–2 | 105 | 22 | 7 | 43 |
| 2–3 | 196 | 52 | 58 | 48 |
| 3–4 | 167 | 81 | 37 | 78 |
| 4–5 | 184 | 90 | 54 | 81 |
| 5–6 | 246 | 96 | 162 | 97 |
| 6–7 | 193 | 97 | 143 | 98 |
| 7–8 | 115 | 100 | 64 | 92 |
| 8–9 | 75 | 97 | 42 | 98 |
| 9–15 | 73 | 100 | 49 | 94 |

* 1 mg. OT, final test.

Total tests: fresh vaccine—1,363; dry vaccine—623.

Total persons: fresh vaccine—1,342; dry vaccine—594.

Comparing the duration of the tuberculin reaction in infants, it was noted that the differences between the dry and the fresh vaccine were negligible, being about 92 per cent at forty-two to forty-seven months for both vaccines.

The rapidity of tuberculin "conversion" and the duration, as well as the quality of the reaction in newborn infants and children following multiple puncture vaccination with fresh or dry vaccine, was similar. These results differ from those reported previously in which there was a definite advantage of the fresh over the dry vaccine. This improvement is related to advances in the methods of culture, harvesting, freeze-drying, and the increased potency of the strain.


## SUMMARY

BCG vaccination when employed as part of a tuberculosis control program can reduce the morbidity and mortality from tuberculosis in infants and children from 68 to 100 per cent as compared with control subjects. In this study it was shown that BCG can prevent tuberculosis in the first one and one-half years of life in successfully vaccinated infants when there has been no exposure to tuberculosis before the vaccination has had a chance to "take." Tuberculosis, when it does occur in a vaccinated host, produces less extensive lesions than in nonvaccinated controls. The amount of exposure to tuberculosis and the caliber of the housing have been found to be important factors in determining the rate of tuberculosis in both the vaccinated and the control infants. BCG should not be expected to do more than other vaccines in situations in which isolation of infected persons is strictly carried out. The multiple puncture method of vaccination has eliminated practically all complications following vaccination. A comparison of fresh and freeze-dried vaccine by newly developed methods reveals that the rapidity of tuberculin conversion and the duration of such are similar for both vaccines.


### *Addendum*

The Institution for Tuberculosis Research of the University of Illinois and the Research Foundation have helped inaugurate tuberculosis control programs of which BCG is a part in 300 projects in 46 states (plus Washington, D. C., and Alaska), including 37 medical schools, 104 schools of nursing, and 230 hospitals. Vaccine is distributed by the Research Foundation. To qualify for receiving vaccine, recipients agree to carry out other tuberculosis control measures, such as no contact with tuberculosis eight weeks before and after vaccination, tuberculin testing, and periodic roentgenograms. The multiple-puncture disc method is used in nearly all of these projects. In many instances comprehensive tuberculosis control programs are initiated for the first time because of the vaccine. A questionnaire was recently sent to some of the physicians who have used BCG regularly for longer periods of time. Those responding to the questionnaire have all indicated that the multiple-puncture method produced practically no complications in newborn infants or children. These programs are all on a service basis. In four projects representing 20,599 vaccinated newborns and children over a four-year period, none report any cases of tuberculous meningitis in the first two years of life. These children come from some of the poorest districts of our large cities.

* * *

Dr. Alway: Thank you, Dr. Rosenthal.

This morning Dr. Middlebrook and Dr. Robinson have accepted the opportunity to give you not the answers to what has been brought out here, but some sort of summarization or synopsis. I was about to say Dr. Middlebrook was going to give us a bird's eye view, but it will be strictly a "Middlebrook view."

Would you give it to us, Gardner?

DR. MIDDLEBROOK: Really, "the problem" is pretty clear. Tuberculosis is a real phenomenon. That's about as far as I can go in defining the problem. Dr. Dubos recognized that in the first remarks he made.

I would like to address myself first to certain problems related to the immunology of tuberculosis. During the course of a brief discussion to emphasize some points that were brought up by various active participants in their presentation of papers which I personally believe should be emphasized, I would like to submit the hypothesis that the mechanism of specific acquired resistance against tuberculous infection or disease is multifactorial.

We have learned that the causation of disease is multifactorial, that the causation of virulence or pathogenicity of microorganisms is always multifactorial. Have we really yet learned that possibly also the mechanisms of acquired resiatance to infectious disease are multifactorial?

I will only suggest this and point out a pertinent observation that Dr. Canetti emphasized. He remarked on the failure of bronchogenic spread in the actively tuberculous human being with open cavity regularly to produce new progressive lesions, and compared with this the enormous susceptibility of the individual who has even previously had tuberculous disease and has an exacerbation from the opening of a small source, small focus, or actually has, as Dr. Mitchell indicated in his recent communication to the *New England Journal of Medicine*, a truly exogenous reinfection.

The experimental animal studies with which I am personally familiar suggest to me that there might not be simply a quantitative difference between the actively tuberculous animal and the animal that has previously had active tuberculosis or has been vaccinated with BCG—an attenuated strain.

There may not be just a quantitative difference between the two. Qualitatively different factors may be involved. In other words, I have been submitting briefly the hypothesis of multifactorial etiology of specified acquired resistance, and in this way suggesting that in future investigations on this subject we not be hypnotized by trying to discover a *single* component of "the tubercle bacillus" which is responsible for all of the phenomena resulting in inhibition of multiplication of tubercle bacilli when they are newly implanted for the first time in a host—guinea pig, rabbit, monkey, or human being.

Dr. Raffel's remarks on agammaglobulinemia, I think, are quite pertinent, as well as Dr. Schick's reference to his patient who really does pretty well without any gamma globulin. My goodness, what have we been taught! What do we teach our associates—that, really, resistance to pneumococcal infection, we all think we know, depends only on one thing, gamma globulin-type specific antibody against the capsular polysaccharide.

There are many, really very many, publications in the literature that suggest that resistance, immunity, to pneumococcal infection and disease is enormously more complicated than that. I would parenthetically like to say that in my opinion the discovery and exploitation of the phenomenon of agammaglobulinemia in human beings is one of the great steps, one of the great advances since 1950, and will offer more opportunities for review of our concepts—maybe reorientation of our concepts—than any other single immunologic affair that has happened in this decade.

This is especially true with regard to human infection and disease, it seems to me.

There is something I believe I have a right to emphasize in connection with the problem of infectivity versus pathogenicity, simply because in our laboratories we have had probably more experience with this phenomenon than has ever been previously offered in using the technique of air-borne infection challenge of experimental animals. I am deeply im-

pressed by the ability of certain changes in the host or changes in the parasite to affect markedly what we might call pathogenicity without having such a striking effect upon what might be called infectivity, the ability to initiate multiplication—sometimes infection is initiated with fantastic ease, suddenly to be cut off by mechanisms which are sometimes certainly determined by host variation; and at other times by factors which are determined by differences between parasites.

With regard to Dr. McDermott's presentation, "Dynamics of Antimicrobial Chemotherapy," this gave him an opportunity to ride herd on concepts that I think extraordinarily important. On the contrary, he stuck pretty well to describing observations which have been made in his department. This is a very laudable thing, but he did not discuss to any extent the implications of these observations for antimicrobial therapy of tuberculosis in man.

I would like to see a revision of our basic thinking with regard to chemotherapy at the practical every-day level in the chemotherapeutic management of human tuberculosis. I would like to see the clinician understand that the tool of roentgenography has to be more extensively supplemented by the tool of microbiology because the chemotherapeutic substances that we use are antimicrobial agents. They do not directly affect proliferation of fibroblasts or laying down of calcium. They don't effect direct changes in roentgenograms. So I hope that future public health policy, in general, will be oriented towards spending more money at the practical level in improving our bacteriologic methods for the diagnosis and treatment of tuberculosis.

Dr. McDermott and Dr. Mitchison both produced evidence that these agents are actively bactericidal *in vivo*. Dr. Mitchison has previously published much material from *in vitro* studies. He presented material yesterday on *in vivo* studies in guinea pigs.

The antimicrobial agents, streptomycin, isoniazid, and PAS, are bactericidal for multiplying tubercle bacilli. I emphasize *bactericidal*. One constantly reads in the literature statements by very competent clinicians to the effect that these agents are only bacteriostatic. They get some justification for this belief from the fact, which Dr. McDermott emphasized, that you cannot eradicate all living tubercle bacilli *in vivo*, but this does not necessarily mean that these agents are bacteriostatic because, as he and Dr. Mitchison emphasized, the state of physiologic insusceptibility of the bacilli to the bactericidal activities of these drugs is intimately related to the state of dormancy *in vivo*. To this extent, and perhaps only to this extent, the problem of chemotherapy is complicated even though one has bactericidal agents.

Thus, the limitation of these agents is essentially due to the response of the host—with its direct effect upon the physiology of the parasite. Under the anaerobic conditions prevailing in the tissues of the host, tubercle bacilli are not significantly sterilized by isoniazid or streptomycin. I believe this should lead to more preoccupation with the phenomenon of persistent excretion of drug-susceptible organisms, even when patients are given two drugs. The agent, as Dr. Mitchison has emphasized, to which the first appearing mutants are resistant has important implications for evaluating the efficiency of the action of the other drug.

Thus far in the literature of tuberculosis, at any level—and pediatricians must at certain points learn from the adult phthisiologist—there has been no really serious discussion of this problem: the persistent excretion of drug-susceptible organisms, or the somewhat more sophisticated, but related, problem of which mutants appear first during the course of chemotherapy.

Dr. Alexander did point out that in tuberculous meningitis drug-susceptible organisms often were isolated during the course of treatment, and she also implied by her conclusions that the problem often was not so much a problem of insusceptibility of the parasite at a genetic level, but insusceptibility of the parasite at a physiologic level.

With regard to Dr. Cocchi's paper, which we loved, I would like to emphasize that cortisone and ACTH may have a mechanical effect on the response to chemotherapy probably only at one level, only in one sense—perhaps only at the bronchocavitary junction—and

that the rest of its effect as an adjuvant, when it acts as an adjuvant in the chemotherapy of tuberculosis, is primarily a biochemical one. His observations on the effect of cortisone in tuberculosis of the central nervous system certainly indicate that these hormones are useful if integrated well—perhaps not by quite so high-powered a multifaceted attack as Dr. Cocchi likes to use—but integrated well with careful control studies. He did not present many cases, but even I sometimes believe that you don't always need contemporaneous control studies. A few patients studied well are worth more than a thousand patients inadequately studied.

Dr. Lack emphasized a problem that is also intimately related to this problem of microbial dormancy. That is the problem of what happens to the bones of human beings when they are immobilized. I really should not speak; it should be Dr. Dressler who should speak on the problem of the integration of bed rest, rest, immobilization, and so on, with chemotherapy.

Suffice it to point out something that I think is of historical interest, pertinent to the present problem: the common-sense notion that if *A* is good, and *B* is good, *A* plus *B* must always be better. Is this necessarily true?

Many of you will remember that during the second decade of this century there was a great resurgence of interest in diabetes mellitus from the point of view of what one could do metabolically with diabetics. Metabolism began to filter into the clinic; and the practice of starvation, undernutrition, became quite a widely accepted one in the treatment of diabetes mellitus. It saved many lives. We are certain that this was a lifesaving practice in many instances.

Then in 1921 along came insulin, and I will only quote from Joslin's report on his studies with insulin in his book published two years later: "Undernutrition . . . is the foundation stone of diabetic treatment. Insulin utilizes rather than replaces the advances . . . hitherto achieved." (*Treatment of Diabetes Mellitus*, Lea & Febiger, Philadelphia, 1923, pp. 489–490).

Dr. Rist isn't here. I want briefly to discuss the problem of the significance of isoniazid-resistant mutants because of my close relationship to some of the experimental work which has been carried on in this field.

I am glad Dr. Rist isn't here because this gives me an opportunity to express my enormous admiration for the ability (and this doesn't exclude you, Georges Canetti, or you, Dr. Debré) of a French scientist to come to this country and present such a lucid paper. I think he is even better acquainted with the use of the term "cavity" than you were, Georges.

We, at this institution, have been concerned with the pathogenicity of these organisms for the tuberculous patient as well as for experimental animals. You will note that we have not published anything concerning our opinions on the epidemiologic significance of these organisms. This is because of our conviction that there is a great deal of difference between pathogenicity and infectivity of microorganisms. I think that, if you will come to the laboratory, we will certainly be able to show you photographs (we don't actually have animals available at the moment) which will prove to you that even the isoniazid-resistant mutants that are catalase negative are highly infective by air-borne infection for the guinea pig, the animal for which they are commonly considered "avirulent"—a word which I think is most inappropriate. I think the word "avirulent" is most misleading both at the experimental level and clinically in discussions of this problem.

In the guinea pig, rupture of a necrotic focus in the lung into a bronchus and evacuation of its contents is, as Dr. Rist pointed out, an extremely rare event. In man it is a common event. Many of the studies on primary tuberculosis in infants and children clearly point out that the incidence of positive sputum and positive gastrics is very high during primary infection, and that the vast majority of individuals excreting such organisms then turn sputum negative and 80 or 90 per cent fail to have any manifestation of progressive disease either at that time or before they reach the age of twenty, as Dr. Debré pointed out.

So, I think the high infectivity of these organisms, even though they are probably unable to manifest their full pathogenicity in human beings, should make one consider these organisms still a public health menace to that degree and should remove the perhaps too

widely accepted notion that patients who are excreting these highly attenuated organisms don't have to stay in a hospital—that they are not going to cause any trouble.

Perhaps these organisms in many individuals will produce a primary lesion with central necrosis which won't excavate, or, if it does excavate, you might not know it unless you do a tuberculin skin test.

I think it is very important for all clinical investigators in tuberculosis to continue, as Dr. Rist suggests, to look for cases of primary infection and particularly disease, whatever the extent of that disease be, in human beings with isoniazid-resistant mutants of tubercle bacilli, and in particular that category of isoniazid-resistant mutants which are highly attenuated in the guinea pig; specifically, the catalase-negative mutants.

There is a point that Dr. Schmidt brought out which is pertinent to this whole problem. He and all of us observe a distribution curve of degree of limitation in pathogenicity for catalase-deficient strains. This distribution curve should not surprise us, as Major Morse, if he is here, will agree, because he and I have talked at length on the variability and degree of pathogenicity of ordinary strains of tubercle bacilli. They are not quite so uniform as we used to believe. This is another reason why the term "the tubercle bacillus" really isn't appropriate when one is discussing such problems as why these mutants should vary so much. They vary markedly in their infectivity. Whether they vary so much in their pathogenicity is another problem, but there again one has to consider variations in the host, which are of equal importance in all infectious disease problems.

With regard to dosage of drugs, I think we have covered that pretty well. It has come up at various times during these sessions. It is evident that we need in tuberculosis today a pharmacologic as well as a microbiologic approach at the experimental level and, certainly, at the clinical level.

We know all too little about the metabolic disposition of isoniazid, or of PAS for that matter (which is a much older drug), in infants and children. We know already that there is great variability from one adult human being to another in the blood level which is achieved and maintained after standard doses of isoniazid. Thus I think, instead of closing our minds to the problem of dosage, we should stimulate further investigation of the pharmacologic and metabolic disposition of isoniazid and other antimicrobial agents in tuberculosis.

With regard to chemoprophylaxis, Dr. Canetti, I think your suggestion is extraordinarily perceptive. I don't think it was quite understood. You had to express it again yesterday, I believe.

I think that the suggestion that one might be able, during the period of conferring specific acquired resistance by vaccination, by whatever means you wish, on human beings, at the same time to protect them from exogenous infection with short-term isoniazid treatment is a concept not only applicable in tuberculosis which would be an immediately profitable route to investigate, but more widely applicable in the future—perhaps in the control of other infectious diseases.

I can only say that we are already doing studies on the relative immunogenic capacity of the isoniazid-susceptible and the isoniazid-resistant mutants of the Phipps BCG strain. Actually the results of the first experiment were available this morning and they may be seen in our laboratory this afternoon. The guinea pig lungs are out for demonstration purposes. If anyone is interested in what immunization with BCG does in guinea pigs and how it manifests itself on air-borne infection challenge, I invite you to look at the lungs of these guinea pigs, the controls, those vaccinated with the BCG catalase-negative mutant strain, and those with the ordinary BCG strain. Fortunately, we succeeded in injecting exactly the same number of bacterial cells of each strain into the animals. There are 20 guinea pigs in each group. I am sorry there were not 200 or something like that; but for our purposes we didn't need that many. Only 5 of each group have been sacrified now. Today is twenty-nine days after challenge.

We want to see what happens with the remaining 15 pigs in each group over a longer period of time. I must say that the only drawback I can think of in regard to your sugges-

tion, Dr. Canetti, is the fact that isoniazid-resistant mutants, *catalase positive*, which in many cases do not manifest, we may say, such highly attenuated pathogenicity, may be in the environment of these people. You might miss protection in that case—you might get superinfection and disease with such organisms. This slight objection is characteristic of all problems when they meet the light of practical public health application.

DR. ALWAY: Thank you, Gardner.

Dr. Canetti, isn't it nice that he only takes on opponents who can win!

We may have lost sight occasionally, during the course of this Symposium, of what actually started us off on it, and that was the fact that in our regular Wednesday morning pediatric tuberculosis rounds there was confusion compounded more often than not. Because of my only contribution as a cockleburr under the saddle, and Dr. Middlebrook's and Dr. Robinson's willingness to be goaded, Dr. Robinson came up with the idea of the Symposium.

I am now going to ask him to be somewhat pragmatic, to share with you the practitioner's evaluation and application of the material presented, which we trust many of the auditors will be able to carry away with them.

DR. ROBINSON: Dr. Alway, Ladies and Gentlemen:

As Dr. Middlebrook said, tuberculosis is a problem. During the past few days you have heard many conflicting opinions, many admissions of our ignorance on basic and pertinent matters, and the multiplicity of new problems which are constantly confronting us. All of this is as it should be.

Progress is made by studying failures rather than by dwelling on successes. However, speaking now as a practitioner of pediatrics faced tomorrow, let us say, with a child with tuberculosis, what have I gained from these discussions?

First, and it may seem unnecessary to say this to this group (I think it is necessary to say this to many pediatricians, however), we know that there is no validity to the oft-repeated statement that tuberculosis is no problem in childhood, and that anyhow most children with it get well. We cannot be satisfied with the "most." The parent whose child has tuberculous meningitis is not interested in the number of children with primary infection who get well.

I am convinced that tuberculosis will be found in children wherever and whenever it is diligently looked for. This is by way of making a plea for the routine use of the tuberculin test by all pediatricians, in order to pick up children with tuberculosis and to track down the adult contact who was the cause of the infection.

Next comes the question of BCG. We are all impressed by the absence of cases of tuberculous meningitis in children properly vaccinated. This, it seems to me, is of itself ample justification for the vaccination of all children who are exposed to a tuberculous milieu, particularly to parents or relatives with any history of active tuberculosis.

Then there is the problem which will also face me tomorrow, when I see a child with primary tuberculosis. The management of this child at the present state of our knowledge is full of question marks. I think the pediatrician faced with this problem right now must be greatly influenced by host factors.

Many of the discussers have stressed the importance of age, nutritional status, recency of conversion of the tuberculin test, as influencing one's approach to management. I should like to add to this list the interest of the parents in the child and the amount of tuberculosis in the family background.

I purposely omit what specific therapy I would use, if I decided to give it, since this is a matter for much disagreement. We would all agree, I think, that isoniazid in "adequate doses" should be part of this therapy, particularly in view of our widespread impression, not yet absolutely proved, that this may prevent the sequel of tuberculous meningitis.

Whether or not other complications of primary disease will also be prevented, a long-term follow-up of such studies as Dr. Debré's and the Public Health Service study will have to tell us.

I think, however, we should keep in mind the thought that, although we may be ad-

ministering isoniazid to the patient in adequate doses, effective concentrations of the drug may not, for one reason or another, be killing the tubercle bacilli they are aimed at.

We have heard the evidence that steroids, in combination with antimicrobials, may make the treatment of certain forms of primary and progressive primary disease much more effective.

The efficacy of present modes of antimicrobial therapy of tuberculous meningitis, with and without steroids, has been discussed. The problem of block seems to be getting less serious. I must confess to having been greatly impressed by Dr. Terplan's pictures of cerebral infarction, and wonder if we are not rapidly approaching an irreducible minimum in our mortality and morbidity rates from established tuberculous meningitis. Certainly cortisone, isoniazid, and streptomycin cannot cure and may not even be able, per se, to prevent these permanent changes.

The common problem of endobronchial disease was touched upon. We seemed to be agreed that bronchoscopy, at least in selected cases, is therapeutically useful and that antimicrobial therapy is desirable, although we were not all impressed by its efficacy in customary doses.

In the discussion on bone tuberculosis, the question of more serious complications actually arising from the immobilization rather than from the bone tuberculosis itself was brought up. There seems to be a general disposition to diminish emphasis on bed rest in bone tuberculosis, and indeed in all forms of tuberculosis in children.

Rehabilitation in adults was treated by Dr. Dressler. I should like to submit that rehabilitation of children is just as, if not more, important.

The problems raised by removing infants and small children from their homes for long periods of time, with the attendant effects upon the children *and* on the parents, the effect of this disease on the sensitive personality of the adolescent—all of these are vitally important considerations in the management of young patients. Perhaps group therapy should be instituted for the parents of these children and for the older children themselves, as is being done now in other chronic diseases, particularly in nephrosis and rheumatic fever.

In conclusion, this all emphasizes the necessity for reiterating, with Drs. Dressler, Lincoln, Sifontes, Johnston, and Lack, that the pediatrician is constantly faced with the problem of treating the child with tuberculosis, *not* tuberculosis in the child.

DR. ALWAY: Thank you, Dr. Robinson.

I have here a terrifically long list of questions as yet unanswered, which have been given to me over the last several days. We could stay here and answer many of them, or hear that there are no answers to most of them; but I rather suspect that what Dr. Schick mentioned yesterday, "meetingitis," is beginning to get us. That of course is a reflex phenomenon affecting cerebral function and coming from an irritating focus, so we won't indulge in pursuing all of these unanswered questions, even though I have been told several times this week that the Symposium Committee would be castigated, if not worse, if all questions were not answered.

I think, however, before we close, it would be proper, as well as very nice, to give our participants, who have already given us so much, an opportunity to make some final statement, should they so choose, on something they feel has been misrepresented or otherwise, if the spirit so moves.

DR. DEBRÉ: In the name of our foreign guests, I would like to make a few remarks. Fortunately for me, I suppose, I am the oldest of your foreign guests. We have come from different countries—from Norway, Sweden, England, India, Greece, Italy, France, and other countries—and I have the pleasant duty to say in their name how satisfied we are with your delightful hospitality, and how satisfied we are with the important lessons learned during this meeting.

Sometimes it was a little confusing in our minds, but not because it was a measure of experience in guinea pigs, monkeys, and other animals than the human one. The confusion for me lies in the difficulty I have in understanding every word of English. I hope I will find

everything in the reports. I have only a charming, useful, excellent impression of this perfect meeting.

I would ask you, Mr. Chairman, to transmit to the President and Trustees and medical staff, and other personnel of the National Jewish Hospital at Denver, our greetings and thanks.

Now I have a duty to give to Dr. Middlebrook a small souvenir of the meeting he and his associates organized. In the name of the International Children's Center of Paris, I have the great pleasure to give to Dr. Middlebrook a small souvenir. It is a medal, the reproduction of an old medal from the Eighteenth Century, made in our National Medal Institution in France, founded seven centuries ago by the King of France.

And this medal gives the picture not of a scientist, a medical man, a biologist, a physician, but of Voltaire. You know the man, one of the most spirited men of the Eighteenth Century. At a time of trouble in England and France, preparing for modern ideals, Voltaire was fighting during his long life for freedom and justice and against inequality between different groups of men, the different kinds of slavery, and for the rights of man all over the world.

I hope Dr. Middlebrook will accept this plaque of Voltaire as a souvenir of this wonderful international Symposium.

DR. MIDDLEBROOK: Thank you very much, Dr. Debré. I would like to say a few words, if you will sit down, about some changes in intellectual climate since *The Age of Reason*.

I am a great admirer of Voltaire, but there is something which in my training has led me to believe that some improvement in man's approach to justice, to permanent peace, to good health for all—not just the absence of disease, but good health—has taken place since Voltaire's time.

In particular it seems to me that the great intellectual failing of "les encyclopédists" was that they did not take into consideration the fact that human beings have to be motivated emotionally. Dr. Dressler emphasized during the course of this Symposium that human motivation is an important aspect of medical or public health treatment of human beings regardless of the nature of the somatic disease process. Indeed, I would predict that the most important problems facing us in the use of drugs for the prevention or treatment of infectious diseases and in particular of chronic disease, infectious or noninfectious, are problems of human motivation.

I am looking at Dr. McDermott, and I will simply recount a little comment that he once made. He considered it rather an aside, but I think his experience was so important. He happened to be taking a drug not long ago, and he said, "You know, I have the hardest time remembering to take that drug every day or twice a day."

I think that perhaps we can agree that we should have learned something since "les encyclopédists" with regard to the motivation of human behavior, of not becoming so "rational" in our approach that we are deceived into believing that, since we have a therapeutic tool, it will be automatically applied successfully.

Thank you very much, Dr. Debré.

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The meeting adjourned *sine die* at twelve-thirty o'clock.